VOLUME 16 NUMBER 6

# NUTRITION REVIEWS

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# NUTRITION REVIEWS

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## PYRIDOXINE AND CEREBRAL ACTIVITY

Each vitamin of the B-complex is essential to the proper functioning of the nervous system. This is due to their requirement as coenzymes for enzymatic steps which are critical in the metabolic economy of neural tissues. It has not always been clear how interference at such steps results in particular signs and symptoms, since many of the details of metabolic interrelationships still await elucidation. Pyridoxine and its related members of the Vitamin $B_6$ group have been an example of this situation, but now it is possible to assemble several lines of evidence into what promises to be a meaningful synthesis.

Pyridoxine holds an unique position in the B-complex. It is the only member for which deficiency or interference with its function commonly result in epileptiform seizures in all mammalian species, including man (D. B. Tower, *Am. J. Clin. Nutrition* **4,** *329 (1956)*). The characteristics of the seizure state, as seen in pyridoxine-deficient human infants, are strongly indicative of a biochemical lesion (*i.e.*, a functional interference with an essential metabolic step which can be promptly reversed or corrected by appropriate measures). The relief of clinical seizures and return of electroencephalograms to normal within minutes after administration of intramuscular pyridoxine to such infants (D.B. Coursin, *J. Am. Med. Assn.* **154,** *406 (1954)*) can scarcely be explained on any other basis. The affected metabolic system may be expected to be intimately concerned with processes regulating neuronal activity.

The number of metabolic steps potentially concerned is large. Pyridoxine, in its coenzyme form of pyridoxal phosphate, catalyzes a formidable array of reactions involving amino acids and related compounds. Essentially these fall into two broad classes, the transamination reactions and the decarboxylation reactions. In this latter group there is one pyridoxal phosphate-dependent reaction which is unique to the central nervous system. It is the sequence by which glutamic acid undergoes decarboxylation to form $\gamma$-aminobutyric acid, which in turn loses its amino group (by transamination with $\alpha$-ketoglutaric acid to reconstitute glutamic acid) to leave succinic semialdehyde, which is readily converted to succinic acid (E. Roberts, *Prog. in Neurobiol.* **1,** *11 (1956)*). Both the decarboxylase and the transaminase are pyridoxal phosphate-dependent, and the decarboxylase and its product, $\gamma$-aminobutyric acid, are found only in central nervous tissues, principally gray matter. Pyridoxal phosphate dissociates easily from the decarboxylase apoenzyme, so that the latter is highly susceptible to pyridoxine deficiency or to conditions which inactivate or interfere with pyridoxal phosphate function.

Initially the role of this system in the metabolic organization of the central nervous system was unknown. In effect it provides a by-pass around the Krebs cycle step from $\alpha$-ketoglutaric acid to succinic acid, which is an important energy-yielding reaction. The significance of the "by-pass" has recently become more apparent. The inhibitory factor, Factor I, isolated from mammalian brain, which inhibits various synaptic reflex preparations has been identified as $\gamma$-aminobutyric acid (A. Bazemore *et al.*, *Nature* **178,** *1052 (1956)*), and the effects of the latter on peripheral and central synaptic activity have led to the suggestion that $\gamma$-aminobutyric acid may function as a moderator or regulator of neuronal activity (T. Hayashi *et al.*, *Abstr. Comm. XX Int. Physiol. Congr. 410 (1956)*; C. Edwards and S. W. Kuffler, *Fed. Proc.* **16,** *34 (1957)*; K. Iwama and H. Jasper, *J. Physiol.* **138,** *365 (1957)*). The neurophysio-

logical role of γ-aminobutyric acid is not yet clear, but these preliminary observations appear to bear upon the problem of seizures and the relation of pyridoxine thereto.

The close connection of pyridoxine with seizure mechanisms is indicated by a number of examples. Experimentally induced pyridoxine deficiency in animals commonly is accompanied by seizures as a cardinal sign of the deficiency. The human counterpart has already been mentioned. In addition several infants with "pyridoxine dependency" have now been reported (A. D. Hunt *et al.*, *Pediatrics* **13,** *140* (*1954*); O. A Bessey *et al.*, *Ibid.*, **20,** *33* (*1957*)). These youngsters require extra daily pyridoxine to control their seizures and are considered to represent examples of disordered pyridoxine metabolism (presumably related to faulty absorption, utilization, degradation or combinations thereof).

Interference with proper functioning of pyridoxine also commonly results in seizures. The pyridoxine antimetabolites, such as deoxypyridoxine or methoxypyridoxine, have been shown *in vitro* to compete with pyridoxine for the appropriate apoenzymes; and the hydrazides, such as semicarbazide, thiosemicarbazide and isonicotinyl hydrazide (Isoniazide®), form hydrazone complexes with pyridoxal or pyridoxal phosphate to inactivate them effectively. (These complexes have been isolated from brain tissue and from urine after experimental administration of hydrazides *in vivo*). In each case seizures occur both in experimental animals and in man. It is interesting that such interferences with pyridoxine function appear to involve γ-aminobutyric acid as well. Hydrazide-induced seizures are associated with decreased levels of cerebral γ-aminobutyric acid and decreased activity of glutamic decarboxylase, both of which can be restored to normal by addition of pyridoxal phosphate (K. F. Killam and J. A. Bain, *J. Pharm. Exp. Therm.* **119,** *255, 263* (*1957*)). Control of such seizures can be effected not only by administration of pyridoxine but also by administration of γ-aminobutyric acid for both hydrazide-induced seizures and deoxypyridoxine-induced seizures.

A connection between these observations and those for certain other types of seizures can now be suggested. In human epileptogenic cortical foci, there is a significant decrease in glutamic acid during incubation *in vitro*, whereas control samples regularly show a significant increase under identical experimental conditions. This decrease in glutamic acid is a "metabolic loss" within the tissue (*i.e.*, some abnormal demand for, or utilization of, glutamic acid). A similar situation is encountered with incubated cortical samples from animals in which seizures are induced by methionine sulfoximine or by 3-methyl-3-ethylglutarimide (Megimide®). In all three instances glutamic acid metabolism can be restored to normal by *in vitro* addition of γ-aminobutyric acid or its lactam, 2-pyrrolidinone (D. B. Tower, *Clin. Chim. Acta.* **2,** *397* (*1957*)). In Megimide®-intoxicated samples the decrease of glutamic acid under these conditions is profound and is clearly associated with a marked decrease in γ-aminobutyric acid. Two other convulsants, fluoroacetate (R. M. C. Dawson, *Biochem. Biophys. Acta.* **11,** *548* (*1953*)) and strychnine (C. Haber and L. Saidel, *Fed. Proc.* **7,** *47* (*1948*)), are also reported to produce decreases in cerebral glutamic acid levels. Since glutamic acid is the precursor of γ-aminobutyric acid, it is possible that these metabolic disturbances may contribute to seizure activity in a manner analogous to those associated with various types of pyridoxine deficiency. Failure of glutamic acid supplies, dysfunction of the decarboxylase itself, or abnormal demand for, or utilization of, γ-aminobutyric acid could singly or in combination achieve the same end result, a level of γ-aminobutyric acid inadequate to "regulate" neuronal activity at its proper level.

Evidence cited to this point argues strongly for the importance of the glutamic acid - γ-aminobutyric acid system in neu-

ronal activity and for an intimate relationship between dysfunction of this system and the occurrence of seizures. Experimentally this view is supported by observations *in vivo* that seizures induced by 3-methyl-3-ethylglutarimide or by pentylenetetrazole (Metrazole®) can be prevented not only by premedication with standard anticonvulsant drugs but also with γ-aminobutyric acid and its lactam, 2-pyrrolidinone (J. E. Hawkins, Jr. and L. H. Sarett, *Clin. Chim. Acta.* **2,** *481 (1957)*). Comparable results have been obtained clinically in a preliminary small group of patients.

It is not yet justifiable to conclude that such *in vivo* results are necessarily related to experimental and *in vitro* observations. In addition it is not clear how any of these findings relate to other data on seizure mechanisms, such as defects in acetylcholine and electrolyte metabolism, and the *in vitro* and clinical effects of L-asparagine (D. B. Tower, *Neurology* **5,** *113 (1955)*), to action of other convulsant agents, or to the mechanisms of action of anticonvulsant drugs, some of which are known to affect electrolyte metabolism (D. M. Woodbury, *J. Pharm. Exp. Ther.* **115,** *74 (1955)*), but none of which has yet been related to γ-aminobutyric acid metabolism.

The data which have been discussed are of additional interest to the field of nutrition in view of the therapeutic trials resulting from them. One set has been aimed at attempting to correct the abnormalities in glutamic acid and/or γ-aminobutyric acid metabolism, using chronic, oral administration of relatively large doses of L-glutamine, L-asparagine, γ-aminobutyric acid, and its lactam, 2-pyrrolidinone. The doses are of the order of 2 millimoles per kilogram body weight four times daily (or some 50 to 60 g. per day for an average adult). These studies have demonstrated the feasibility of long term administration of these compounds to man without significant toxicity or disturbance of nutrition. A possible exception is γ-aminobutyric acid, since in man it produces acute numbness and tingling of the extremities, a sensation of nausea or dizziness, and subjective and objective flushing, only the last of which persists on chronic administration. In part as a result of these studies, pure, synthetic L-glutamine and synthetic γ-aminobutyric acid have become available for nutritional and other studies.

A second aspect concerns the indications for, and value of, employing pyridoxine as an anticonvulsant. This subject, as it relates to young children, has been recently reviewed by O. A. Bessey *et al.* (*Pediatrics* **20,** *33 (1957)*). Several trials in older age groups have been reported, all but one being in the negative. There are few means at the disposal of the clinician to determine which seizure patients would benefit from pyridoxine therapy. The excretion of xanthurenic acid after a tryptophan load is considered the most sensitive indicator of pyridoxine deficiency, but even this has been found to present some complexities in the patients reported by O. A. Bessey and colleagues. The determination of levels of circulating, or available, pyridoxine and its derivatives should offer a more direct approach. It may now be more feasible with combined chromatographic and fluorimetric methods recently reported (A. Fujita *et al.*, *J. Vitaminol.* **1,** *267 et seq (1956)*). Certainly such data would be of basic value, since the very few available studies indicate considerable lability in blood and cerebrospinal fluid levels. There is an additional consideration of potential importance here. In relatively massive doses pyridoxine is toxic, producing signs and symptoms similar to those resulting from its deficiency (D. B. Tower, *Am. J. Clin. Nutrition* **4,** *329 (1956)*). The indiscriminate use of pyridoxine, particularly in infants, carries a potential danger which could be obviated by preliminary determination of the state of pyridoxine nutrition. For this, suitable control data for blood and urine must be available.

Discussion has been restricted to the area of cerebral activity, but pyridoxine is

also concerned with other aspects of nervous system function. Deficiencies or interference with activity of pyridoxine produces neuropathies and myelopathies in many species, including man. These dysfunctions may relate to the metabolic areas already considered, or perhaps more directly to lipid metabolism, which is also dependent to some extent upon pyridoxine activity. Perhaps most significant of all, deficiencies of pyridoxine during gestation can result not only in a convulsing infant but also in one with severe ataxia and spasticity, indicating involvement of many other neural structures and systems (W. J. McGanity, *Am. J. Clin. Nutrition* **4,** *376 (1956)*). The possibility that the converse, excess pyridoxine during gestation, may also adversely affect the fetus is suggested by the seizure case studied by A. D. Hunt *et al.* (*Pediatrics* **13,** *140 (1954)*). These are isolated observations in an area which needs much more detailed investigation.

Three general conclusions may be drawn. Pyridoxine is important as a coenzyme for normal metabolism and function of the central nervous system. In specific seizure situations pyridoxine "nutrition" is the critical factor. And for seizure mechanisms in general, pyridoxine function in neural tissues has focused attention on metabolic areas which may be significantly involved in such mechanisms. The evidence in regard to the glutamic - $\gamma$-aminobutyric acid system appears to be highly suggestive, but it is not sufficient to permit a complete outline of a potential mechanism which would account for the initiation of cerebral seizures. This is a combined neurophysiological, neuropharmacological and neurochemical problem. The foregoing data should assist materially in determining the direction of further attacks upon its solution.

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## PROGRESS IN PARENTERAL NUTRITION WITH FAT EMULSIONS

The clinical need for a stable, safe fat emulsion for parenteral administration has long been recognized. Clinicians have been frustrated in their attempts at maintaining nutritional balance via the parenteral route by their inability to provide enough calories by the administration of sugar and amino acid solutions intravenously to permit nitrogen balance. Attempts have been made for nearly 40 years to perfect fat emulsions for intravenous use. The preparations used by J. M. McKibbin *et al.*, (*J. Lab. Clin. Med.* **30,** *488 (1945)*) represented a considerable advance over those used by earlier investigators. Soya phosphatides (lecithin) with a small amount of a co-emulsifier were used to stabilize the fat emulsions. Infusions of these fat emulsions into patients were without incident in 70 to 80 per cent of cases. The remaining patients, however, exhibited undesirable side effects such as back pain, fever, nausea and vomiting.

A vast amount of clinical experience with a new fat emulsion has been published in a special symposium in *Metabolism* (**6,** *591 (1957)*). The composition of this emulsion is described by C. E. Meyer, J. A. Fancher, P. E. Schurr and H. D. Webster (*loc. cit.*). Fifteen per cent cotton seed oil is stabilized in an homogenized form with highly purified soya lecithin and a nonionic co-emulsifier. The emulsion also contains 4 per cent dextrose. Usually 600 ml. of emulsion providing 960 calories are given with each infusion in man. Chronic toxicity studies of the emulsion and its separate constituents were carried out in dogs. The only apparent change after 30 infusions was the accumu-

lation of brown pigment in the cells of the reticuloendothelial system of all 16 dogs studied. Microgranulomas were found in the livers of dogs after repeated infusions of this fat emulsion. The latter lesions have not been observed by the authors in liver biopsy and necropsy studies in humans receiving this product.

The effects of this emulsion on nitrogen excretion in dogs during attempts at complete parenteral alimentation are described by R. S. Levine, E. C. Calvary, J. E. Plzak and J. G. Allen (*Ibid.* **6,** *597 (1957)*). Depletion was induced by drastic caloric restriction and the ability of various types of oral and parenteral regimens to restore nitrogen balance was observed. It was found that worthwhile nitrogen retention occurred only with adequate caloric replacement with the fat emulsion (60 calories per kilogram). The retention of nitrogen was more satisfactory with plasma than with the protein hydrolysates as sources of amino acids. Anemia developed in the dogs on parenteral alimentation but did not seem peculiar to the fat emulsions, occurring with glucose and fructose infusions also. The anemia was most pronounced with plasma as a source of amino acids, probably due to expansion of plasma volume.

Very careful metabolic studies of normal and malnourished human beings receiving this fat emulsion are reported by H. L. Upjohn, M. C. Creditor and S. M. Levenson (*Ibid.* **6,** *607* (1957)). Under a variety of experimental situations the authors demonstrated that the caloric availability of intravenous fat emulsions was indistinguishable from oral fat. Studies of electrolyte metabolism during fat emulsion infusions demonstrated no significant changes although sodium and potassium diuresis followed the intravenous fat emulsion periods in some cases. Erythrocyte survival was found to be normal in the three cases in which it was studied after several infusions of fat.

The symposium records the experience of a number of clinics with this parenteral nutrient. In general there is considerable agreement about the effectiveness of the fat emulsion in providing metabolically assimilable calories. Despite the improvement in the stability and the incidence of reactions, side reactions were observed by all authors. The findings of R. C. Bozian, N. W. Davidson, L. J. Stutman and C. F. Wilkinson (*Ibid.* **6,** *703 (1957)*) may be cited in this regard. An earlier preparation of the fat emulsion produced fever, chill, altered blood pressure, back and bone pain, nausea, and vomiting in approximately 10 to 15 per cent of the cases. The more purified later products had a far lower incidence of these immediate reactions. It was noted, however, that the occurrence of fever after the infusion was as common with the newer preparation (15 per cent) as with the older one (16 per cent). Because it had previously been observed that individuals who developed delayed fever cleared their blood more rapidly of fat than those not developing fever in most instances, the effect of prolonging the period of infusion of 600 ml. of fat emulsion from three and one half hours to seven hours was observed by A. L. Forbes, (*Ibid.* **6,** *645 (1957)*). Fever occurred as frequently with slower rates of infusion. Repeated infusions are associated with fewer delayed febrile reactions. Considerable doubt still remains about the mechanism of this fever. Arguments are cited suggesting that it may be due to an unrecognized pyrogenic substance, and equally convincing arguments are presented indicating it may be a metabolic response to intravenous fats.

Although most of the reports of the use of this fat emulsion are favorable, evidence of untoward reactions following prolonged administration is recorded. In burned patients B. H. Gaston (*Metabolism* **6,** *735 (1957)*), has observed changes in liver function. Increasingly abnormal liver function tests were found in a group of patients with portal cirrhosis receiving multiple infusions of fat

emulsion by F. Kern, Jr., R. G. Jackson, T. E. Martin and J. F. Mueller (*Ibid.* **6,** *743* (*1957*)). It is interesting that in these patients hepatic biopsies failed to show progressing damage. A more severe toxic reaction was observed by D. M. Watkin (*Ibid.* **6,** *785* (*1957*)) in a patient after 20 infusions of fat emulsion, characterized by anorexia, abdominal pain, sore throat, headache, enlargement of liver and spleen with jaundice, fever, anemia and thrombocytopenia with bleeding. Two similar cases are reported by S. M. Levenson, H. L. Upjohn and T. W. Sheehy (*Ibid.* **6,** *807* (*1957*)) in whom hemolytic anemia and bleeding tendencies developed.

From the many articles in this important symposium it is apparent that real progress has been made in the development of useful parenteral fat emulsions. The present preparation can be used safely over short periods of time. It would appear that its use for greater than two weeks would be associated with increasing hazard. There is reason to hope the final solution to this problem will not be long delayed and complete parenteral nutrition will be practical over extended periods of time.

## FOLACIN METABOLISM IN SPRUE

The disease sprue is characterized by diarrhea, glossitis, weight loss, anemia and megaloblastic marrow. There is generalized malabsorption from the gastrointestinal tract. Not only the absorption of fat and fat-soluble vitamins is impaired, but also the absorption of glucose (*Nutrition Reviews* **4,** *147* (*1946*)), water and electrolytes (*Ibid.* **11,** *261* (*1953*); **14,** *173* (*1956*)).

The anemia of sprue suggested the involvement of folacin and it was reported that sprue would respond to the injection of this vitamin (*Nutrition Reviews* **4,** *11* (*1946*)). There is evidence, however, that not all cases of sprue will respond to treatment with folacin (*Ibid.* **6,** *114* (*1948*)). The exact etiology of the disease is still unknown.

The possibility of an impaired absorption of folacin in sprue was pointed out by R. H. Girdwood (*Lancet* **II,** *53* (*1953*)). More recently C. E. Butterworth, *et al.* (*J. Lab. Clin. Med.* **50,** *673* (*1957*)) have reported results demonstrating an impaired absorption of folacin in the disease. Three groups of subjects were studied, individuals with untreated sprue or patients with sprue in relapse, treated sprue patients, and normal hospital personnel or patients without gastrointestinal disease (control group). The sprue patients exhibited the usual clinical signs, including demonstrated malabsorption of two or more test substances (xylose, vitamin A, butter, dietary fat). The treated subjects were patients who originally exhibited the clinical signs of sprue and were treated and maintained with folacin. The folacin maintenance dose for these patients was discontinued for one or more days prior to the administration of test doses of folacin.

These subjects were given 5 mg. of folacin either orally or intravenously. The subsequent five-hour urine specimens and samples of serum drawn one, two, three and four hours after administration of the test dose, were assayed for folacin by microbiological assay. The assay measured both folacin and citrovorum factor.

Following the oral administration of folacin the normal subjects excreted 26 per cent of the dose in the urine, treated sprue patients excreted 24 per cent, while untreated patients excreted only 1.2 per cent. Following intravenous administration of the folacin, the three groups excreted in the urine 40, 39, and 27 per cent of the test dose, respectively. Four other individuals with untreated sprue excreted an average of 0.6 per cent of the test dose of folacin. Three of these patients were treated for one to two months with folacin and again tested.

At this time they excreted in the urine 19 per cent of the test dose.

Serum levels of the folacin following a test dose gave additional evidence of impaired absorption of the vitamin in sprue patients. The serum concentration of folacin in millimicrograms per milliliter two hours after the oral administration of the test dose were: normal subjects, approximately 200; treated sprue subjects, approximately 180; untreated sprue subjects, less than 20. Following its intravenous administration, folacin disappeared rapidly from the blood of all subjects, and there was no evidence of a more rapid clearance by the sprue patients.

In order to estimate tissue concentration of folacin in sprue, the concentration of the vitamin in leucocytes was determined. The average content as millimicrograms per milliliter of leukocytes were: normal subjects, 69.3; treated sprue patients, 81.5; untreated sprue patients, 49.1.

In other experiments urine was chromatographed and bioautographs were prepared. These studies were carried out to determine whether the urine from sprue patients contained a folacin antagonist which would interfere with the microbiological assay. No evidence for an inhibitor was obtained.

The results of these experiments (demonstrating a reduced urinary excretion of folacin following the oral administration of a test dose to sprue patients, whereas sprue only mildly affected the urinary folacin excretion following an intravenous dose of the vitamin) indicate that the absorption of folacin from the gastrointestinal tract is impaired in the disease. The blood levels of folacin following the oral dose support this conclusion. Since in treated sprue cases folacin absorption is normal, the defect must not be a permanent one. It is interesting that this disease, which may be due to folacin deficiency, is characterized by a malabsorption of the vitamin. This is truly a fulminating disease.

## ASPIRIN AND DIABETES

The use of aspirin (acetylsalicylic acid) in the treatment of diabetes was described as early as 1876 by W. Ebstein (*Berl. Klin. Wschr.* **13,** *337 (1876)*). D. J. Ingle (*Proc. Soc. Exp. Biol. Med.* **75,** *673 (1950)*) observed that aspirin suppressed the glycosuria of partially depancreatized rats. M. J. H. Smith, B. W. Meade, and J. Bornstein (*Biochem. J.* **51,** *18 (1952)*), using alloxan-diabetic rats, noted that salicylate reduced glycosuria and blood glucose in the diabetic rat but did not alter liver glycogen content. In normal rats the blood glucose was not altered but liver glycogen was depressed. Several mechanisms could be functioning to produce this effect on carbohydrate metabolism: 1.) increased gluconeogenesis; 2.) increased deposition of muscle glycogen; 3.) increased glucose utilization in tissues; 4.) increased conversion to fat; 5.) stimulation of pancreatic islet beta cells. In contrast to the reports of a blood glucose lowering effect, there are additional studies in which hyperglycemia and glycosuria were observed in cases of salicylate poisoning (M. Gross and L. A. Greenberg *The Salicylates, Hillhouse Press, New Haven, Conn. 108 (1948)*). Thus, the possibility exists that the achievement of a reduction of blood sugar level to normal might depend on a suitable adjustment of the dose.

J. Reid, A. J. MacDougall and M. M. Andrews (*Brit. Med. J.* **2,** *1071 (1957)*) became interested in this effect of aspirin when a male diabetic patient was admitted to the hospital for rheumatic fever therapy. At this time he was given full salicylate treatment. In spite of receiving no insulin, his blood sugar level was normal and urine was free of sugar. One week after discharge from the hospital he had a diabetic glucose-tolerance curve, glycosuria, and hypergly-

cemia. These findings prompted the study of the effect of aspirin in seven diabetic individuals. A constant low-carbohydrate diet was given each patient for about two weeks before and during the administration of aspirin. Aspirin doses of 1 to 1.6 g. were given every four hours, except in the middle of the night. Estimations of serum salicylate levels were done in order to avoid inducing undesirable side effects. Evaluation of the effect of aspirin on the clinical manifestations of diabetes was made by determining blood sugar, urine glucose, ketonuria, glucose tolerance and basal metabolic rate.

Fasting blood sugar averaged 191 mg. per 100 ml. on the day when aspirin administration was started. During the two weeks of treatment the blood sugar level fell to normal, and by the sixth day after aspirin was discontinued the level averaged 136 mg. per 100 ml. The urinary sugar excretion during the preliminary control period averaged 38 g. per 24 hours. Following the treatment with aspirin the glucose excretion dropped progressively, reaching an average of 5 g. per 24 hours by day 14. Glucose tolerance tests carried out before, during, and after aspirin therapy showed uniformly lower blood-sugar curves during the period when aspirin was given. Quantitative estimations of urinary ketones in two patients with positive tests in the preliminary period showed a decrease to normal by the end of the second week of treatment. Weight loss was seen in only one patient during the aspirin therapy. When treatment was stopped, however, all except two patients lost from 1 to 2.7 kg. in the following week.

The disappearance of thirst, polyuria, and pruritis during treatment with aspirin was not surprising since these symptoms are intimately related to hyperglycemia. Due to the intensive nature of the treatment high salicylate levels were reached and serious side effects (intermittent vomiting) developed in two of the patients. Reduction of the dose led to disappearance of the symptoms. Tinnitus (a subjective ringing, roaring, or hissing sound in the ears) was experienced by two of the patients, and dulling of hearing occurred in all, but disappeared when therapy stopped. Determination of basal metabolic rate in four of the patients before, during, and after aspirin treatment revealed a definite increase in response to the drug.

The early use of aspirin in the treatment of diabetes was discontinued because of the development of toxic effects. However, these symptoms apparently result from blood levels that are higher than necessary for the control of hyperglycemia. Aspirin, compared with the sulfonylureas, has an advantage in that it may be given for prolonged periods without the risk of agranulocytosis. In addition, the maximum tolerated doses lowered the blood sugar to normal without producing hypoglycemia.

Further investigation is needed to assess the place of aspirin in the treatment of diabetes mellitus.

## CHOLESTEROL ABSORPTION IN THE HUMAN

A number of studies of human subjects indicate that serum cholesterol may be lowered by the inclusion of unsaturated vegetable oils in the diet as the source of fat (*Nutrition Reviews* **13,** *8, 44 (1955)*; **14,** *107, 327 (1956)*). The possibility exists, however, that this effect may require excessive amounts of unsaturated vegetable oils, not compatible with acceptability, and that such an effect may only be transient. It is not yet clear whether or not the unsaturated fatty acids exert their influence mainly by altering exogenous cholesterol absorption. The fact that cholesterol is synthesized in the body from simple two-carbon units such as acetate (*Ibid.* **15,** *282 (1957)*) suggests that

that deposition of cholesterol in arterial disease may involve abnormal metabolism as well as abnormal absorption of cholesterol. For these reasons the effects of dietary fatty acids of various degrees of saturation must be tested with and without exogenous cholesterol.

In order to determine cholesterol balance in the human subject and to evaluate the effects of lipids on cholesterol absorption and blood level, it is important to know the amount of cholesterol excretion on a sterol and fat-free diet. Such a study was the subject of a report by A. C. Ivy, E. Karvinen, T. M. Lin, and E. K. Ivy (*J. Applied Physiol.* **11,** *1 (1957)*). Synthetic diets without fat or sterol were fed for seven days. Fecal collections were made during the last three days to determine the amount of endogenous cholesterol excretion. The daily digitonin precipitable sterol eliminated was 421 ± 222 mg. per day with a coefficient of variation of 53 per cent. Calculation of the amount of sterol on the basis of body weight gave a value of 5.8 mg. per kg. per day and a coefficient of variation of 37 per cent, indicating that sterol excretion was related to body weight.

The average elimination of sterol in the males was 6.4 mg. per kg. of body weight per day, and in the females was 4.5 mg. per kg. per day. This difference was not considered statistically significant in view of the small number of subjects. The serum cholesterol was determined before the start of the experiment and after seven days on the diet. In six of the subjects the differences did not exceed the experimental error, one subject showed an increase from 330 to 550 mg. per cent, and in the remaining four the cholesterol decreased slightly.

The cholesterol present in the intestine of subjects on a sterol-free diet arises from the bile, intestinal juice and bacteria. Part is reabsorbed in the lymph and the remainder is excreted. In order to determine the extent of cholesterol reabsorption on a sterol-free diet, E. Karvinen, T. M. Lin and A. C. Ivy (*J. Applied Physiol.* **11,** *143 (1957)*) determined sterol excretion on a basal diet containing a small amount of added cholesterol (380 mg.). The average daily cholesterol excretion was 850 mg. which, when corrected for the endogenous cholesterol excretion of 420 mg. on a sterol-free diet, gave 430 mg. If one assumed that this excreted sterol also included 316 mg. of plant sterols present in the diet, then the difference was 114 mg. of the original 380 mg. ingested, or 30 per cent excretion. If endogenous cholesterol were absorbed to the extent of 70 per cent as well, then the 420 mg. excreted on the sterol-free diet regimen would equal 30 per cent of the endogenous intestinal sterol pool, totaling 1400 mg. of which 980 mg. were absorbed.

Using the basal diet containing 380 mg. of cholesterol, Karvinen, Lin and Ivy determined the amount of sterol excretion in human subjects before and after the addition of 1, 3, 6 or 9 g. of cholesterol daily for one week. The average maximum intestinal absorption of cholesterol was 2.0 g. per day. This represented a range from 0.9 to 2.9 g. The maximum of 2.9 g. was observed twice in two subjects during 19 tests at the 6 g. per day level of cholesterol intake. These data indicate that there is a threshold limiting the cholesterol absorption in the human intestine. Serum cholesterol values were not significantly altered in the interval during which extra cholesterol was fed.

In balance studies on rats, K. S. Kim and A. C. Ivy (*Am. J. Physiol.* **171,** *302 (1952)*) observed that intestinal absorption of cholesterol was impaired when palmitic and stearic acid were included in the ration. The possibility that these long chain fatty acids might also be effective in reducing the cholesterol absorption of man prompted the study by E. Karvinen, T. M. Lin and A. C. Ivy (*J. Applied Physiol.* **11,** *8 (1957)*). The diets used contained standard items common to ordinary American menus and consisted of approximately 10 g. of animal fat,

10 g. of vegetable fat, 380 mg. cholesterol, 120 g. protein and 490 g. carbohydrate. Nine subjects served in the palmitic acid experiment and six served in the stearic acid experiment. In the palmitic acid study the basal diet was fed for seven days and then 30 g. per day of palmitic acid were added for seven days. At this point one egg (300 mg. of cholesterol) was added daily for seven days without palmitic acid, followed by being added with palmitic acid for seven days. In the stearic acid experiment the same pattern was followed except that 3.0 g. cholesterol replaced the egg.

Neither palmitic nor stearic acid had any significant effect on the endogenous cholesterol excretion or on the absorption of added cholesterol. Two possible factors may contribute to this effect, (1) the fact that 55 per cent of palmitic and 21 per cent of stearic acid were utilized, and (2) the possibility than an acceptable diet could not be produced containing large enough quantities of these fatty acids.

The search for food additivies capable of reducing the absorption of cholesterol in the human must also be concerned with the acceptability of the ultimate diet produced.

## IRON ABSORPTION IN INFANTS AND CHILDREN

A quantitative study of the absorption of food iron in infants and children utilizing radioactive iron, as described by C. V. Moore and R. Dubach (*Tr. A. Am. Physicians* **64,** *245 (1951)*), has been carried out by J. Schulz and N. J. Smith (*Am. J. Dis. Child.* **95,** *109 (1958)*).

The subjects in these experiments were normal children, aged three months to 15 years. Milk, eggs, and iron-fortified infant cereal were tagged with $Fe^{59}$ by giving high specific activity radioiron to a cow, to chickens, or by adding it to the cereal during manufacture. The feedings were carried out at home after an overnight fast. The stools were collected quantitatively and analyzed for radioactivity until less than 2 per cent of the administered dose per day was found. This was usually accomplished in four or five days.

Absorption of iron was calculated as the difference between the amount of radioactivity administered and that found in the stools. In many of the patients, blood was obtained on the fourteenth day for the determination of radioactivity in hemoglobin. The total hemoglobin mass was calculated from the body weight and the hematocrit, and iron utilization for hemoglobin formation was expressed as per cent of the radioactivity present in the test meal.

Six ounces of homogenized milk tagged *in vivo* with $Fe^{59}$ were fed quantitatively to ten normal fasting children, aged four to 52 months. The average absorption was 9.1 per cent (range 3 to 13 per cent). The addition of tracer doses of $Fe^{59}$ as ferrous sulfate to 6 oz. of homogenized milk gave similar results in another five children. Four iron deficient infants, aged 11 to 18 months, absorbed 11 to 24 per cent of the iron in 6 oz. of milk. Six normal adults given 600 ml. of milk absorbed 2.8 per cent of the iron.

Tracer doses of $Fe^{59}$ as sodium iron pyrophosphate were added to prepared infant cereals fortified at a level of 50 mg. of iron per 100 g. of cereal and fed as 6 g. portions with 2 oz. of whole milk to 17 normal fasting children three to 26 months of age. Mean absorption was 12.3 per cent with variation from 0 to 25 per cent. There were no significant differences between rice, oatmeal, or mixed cereal, suggesting that the higher phytate content of oatmeal did not interfere with iron absorption when this cereal was fed with milk.

A larger number of children were fed eggs physiologically tagged with radioiron. In 52

normal fasting children one to 15 years of age, the absorption of egg iron averaged 8.6 per cent with a range of 0 to 27 per cent. When the iron absorption from tagged eggs was plotted against age, there appeared to be a relationship indicating greater absorption between one and four years of age, less in the five to eight year age group, with occasional higher values appearing in the children over ten years of age. The low percentage of iron absorption by the five to eight year old children was found in all the children of this age group studied. Schulz and Smith point out that it is at this age that hemoglobin per unit of body weight is at its lowest value for the whole growth period.

Comparison of the absorption of radioiron tagged egg, egg with added tagged ferrous sulfate, tagged chicken liver with egg, and tagged egg with milk in infants resulted in the absorption of 11 per cent, 12 per cent, 8 per cent and 5.8 per cent, respectively. Although these differences are not significant it would seem possible that eggs and milk might interfere with iron absorption, or that iron absorption is inversely proportional to the bulk of the feeding. Tagged egg fed in milk to iron deficient children resulted in a mean absorption of 17 per cent as compared to the 5.8 per cent in the normal subjects.

When the amount of iron absorbed in 40 children receiving tagged egg was compared with that appearing in the circulating hemoglobin within 14 days, a mean value of 2 per cent of ingested iron was absorbed that could be accounted for by hemoglobin synthesis. This observation is probably not statistically significant since, in ten subjects, more iron was found in the circulating hemoglobin than calculated from absorption. The authors point out, however, that the error introduced by assuming normal blood volumes in these children is probably no greater than that involved in a single determination. In the iron deficient subjects, however, more iron was absorbed than was used for hemoglobin synthesis in all eight subjects with an average difference of 7 per cent of the administered radioactivity.

The failure to recover more than 1 or 2 per cent of the administered iron in these iron deficient subjects led to some observations in dogs, by R. C. Neerhout, D. H. Armstrong, Schulz and Smith (*Ibid.* **95,** *126 (1958)*), on the fate of recently absorbed food iron. Fourteen-day metabolic balance studies on normal, iron deficient, and iron loaded dogs fed food containing tagged iron were performed, and 95 per cent or more of the absorbed iron was present in the circulating red cell mass 14 days after the isotope feeding. The iron deficient dogs continued to deposit more iron in their tissues than did the other animals, although these amounts were not as great as appeared to be the case in the iron deficient infants.

This study on the absorption of food iron by children using radioactive isotope techniques is comparable with similar work in adults (*Nutrition Reviews* **13,** *292 (1955)*). Infants and children absorb a higher percentage of food iron than do adults. A summary of all 98 balance studies with food iron would suggest that normal children can be expected to absorb about 10 per cent of the iron from many foods. Another major difference from the observations made in adults is that iron deficient subjects absorbed almost twice as much iron from foods as normal subjects. Statistical evaluation of the data suggested that the iron absorption correlated better with the age and serum iron than it did with hemoglobin concentration, but that over 50 per cent of the factors controlling iron absorption were not known. These observations would support strongly the authors' contention that iron deficiency in infants occurs, not because of a defect in the absorption or utilization of iron, but because the amount in the diet is not adequate, even in the presence of greatly enhanced absorption, to meet the extremely variable needs for iron which may occur during growth.

## FRUCTOSE METABOLISM IN DAIRY COWS

The demonstration that the first few steps in the metabolism of glucose and fructose may be distinctly different (*Nutrition Reviews* **12,** *154, 187 (1954)*; **14,** *52 (1956)*) has aroused considerable interest in both the metabolic and nutritional roles of these sugars. Previous studies had suggested that such differences existed and that these might be of importance in the management of diabetic patients and in parenteral feeding (*Ibid.* **11,** *299 (1953)*; **12,** *39, 227 (1954)*). More recent studies have provided evidence of species differences in the metabolism of fructose (*Ibid.* **15,** *375 (1957)*). Recent observations on still another animal, the dairy cow, by J. R. Luick, M. Kleiber, J. M. Lucas and T. A. Rogers (*Am. J. Physiol.* **191,** *90 (1957)*) provide further knowledge concerning the metabolic fate of fructose.

These authors used two mature lactating Jersey cows for their study. They injected uniformly $C^{14}$-labeled fructose or glucose intravenously and determined the amount of radioactivity expired as $CO_2$, the radioactivity of blood glucose and blood fructose, and the radioactivity present in the milk and in various milk components up to 48 hours after injection. They also determined the specific activities of the various products.

Since the carbohydrates were injected intravenously, differences in their metabolism during digestion or absorption (*Nutrition Reviews* **15,** *375 (1957)*) would not have been detected. Within 15 minutes after the injection of uniformly labeled (U-$C^{14}$) fructose, less than 2.5 per cent of the injected radioactivity was present as blood fructose, while 8 to 11 per cent was present as glucose. In contrast, after injection of U-$C^{14}$ glucose, 16.5 per cent of the radioactivity was still present as glucose after 15 minutes. The plasma fructose fell rapidly after injection, to plateau at a very low level in 20 minutes. There was a corresponding rise in blood glucose labeled with $C^{14}$ during the first 20 minutes after the injection of fructose which, as the authors point out, is evidence that blood glucose is a key intermediate in fructose utilization. This is in accord with recent observations on fructose metabolism (*Ibid.* **12,** *154, 187 (1954)*; **14,** *52 (1956)*) in the rat.

Observations on the radioactivity of expired carbon dioxide also indicated that glucose and fructose were metabolized differently. The specific activity of the respired carbon dioxide rose to a maximum within 15 minutes after the injection of fructose, whereas maximum specific activity occurred 30 minutes after the injection of glucose. Also the rate of decrease of $C^{14}$ in the respiratory carbon dioxide was more rapid after fructose than after glucose injection.

The pattern of isotope distribution in the milk products was much the same after glucose or fructose injection. Within 48 hours about 40 per cent of the radioactivity was present in lactose, 5 per cent in the fat, 5 per cent in the albumin, 3 per cent in the citrate and 2 per cent in the casein. About 48 per cent of the injected radioactivity was recovered within 48 hours in the milk after fructose injection, and 56 per cent after glucose injection. In each case the labeling was a little lower with fructose than with glucose.

After glucose injection, 96 per cent of the injected radioactivity was accounted for within 48 hours, about 40 per cent in expired carbon dioxide and 56 per cent in the milk. After fructose injection only 67 to 79 per cent of the injected radioactivity was recovered within 48 hours, about one-third in the respired carbon dioxide and two-thirds in the milk.

The authors conclude that, although fructose is converted quite rapidly to blood glucose in the body of the cow, a portion of the fructose is metabolized by processes not involving blood glucose. This is suggested by the observation that the peak activity in respired carbon dioxide was reached more

rapidly after injection of fructose than after glucose injection, and that only 14 per cent of the activity from the fructose had appeared in the carbon dioxide within three hours, whereas 22 per cent of the activity of injected glucose was respired within this time. The known pathway by which fructose is broken down in the liver to three carbon units which must be resynthesized to give glucose for blood glucose (*Nutrition Reviews* **12,** *227 (1954)*) might account, at least in part, for these differences. The lower total activity in carbon dioxide, the authors suggest, should be attributed to a more rapid entry of fructose into anabolic processes, such as the synthesis of glycogen and milk constituents. However, the specific activities of the various milk products were lower in each case after fructose than after glucose injection.

From a comparison of the specific activity of blood glucose, after fructose or after glucose injection, with the specific activities of the final products in each case, the authors deduce that as much as 50 per cent of the fructose may be metabolized by pathways that do not involve blood glucose as an intermediate. This is a high percentage and merits further investigation. The fact that 25 per cent of the radioactivity remained unaccounted for after fructose injection, whereas almost the entire activity was recovered after glucose injection, also requires further study. Since fructose is converted more readily to liver glycogen than is glucose (Chaikoff *et al., J. Biol. Chem.* **194,** *435* (1952)), and since fructose can become widely distributed as such in body water (*Nutrition Reviews* **12,** *39 (1954)*) it would seem that both of these possibilities should be investigated in order to determine more accurately the extent to which the cow may differ from other species in the way it metabolizes fructose.

## MAGNESIUM DEFICIENCY IN CALVES

Hypomagnesemia has been reviewed by L. K. Blaxter and R. F. McGill (*Vet. Rev. Annot.* **2,** *35 (1956)*). The deficiency has been reported in sheep and cattle, and occurs most frequently shortly after these animals have started to graze on young, quickly growing herbage. In extreme cases the disorder culminates in convulsions which are often fatal. C. W. Duncan, *et al.* (*J. Biol. Chem.* **108,** *35 (1935)* ) were the first to show that calves, fed on milk for an abnormal length of time, also develop hypomagnesemia. Two principal reasons have been put forward to explain the development of magnesium deficiency under these conditions: (1) that it is due to a dietary shortage of magnesium and (2) that some component of milk interferes with utilization of magnesium. R. H. Smith (*Biochem. J.* **67,** *472 (1957)*) recently undertook the elucidation of this phenomenon.

Diets of the calves used for this series of experiments consisted almost exclusively of whole milk with daily additions of 0.4 g. of $FeCl_3 \cdot 6H_2O$, 0.04 g. of $MnSO_4 \cdot 4H_2O$, and 0.04 g. of $CuSO_4 \cdot 5H_2O$ from two months of age, and double these amounts from six months. Vitamin D supplements were added to the diets as aqueous suspensions.

At ages less than five weeks, the calves retained 39 to 54 per cent of the magnesium provided in their diet. They excreted 13 to 34 per cent of their dietary magnesium in the urine. However, hypomagnesemia appeared sooner or later as the calves became older. This was shown to be associated with a progressive decrease in ability to utilize dietary magnesium. The decrease continued until little or no magnesium was retained in the blood or excreted in the urine. Addition to the milk diet of supplementary inorganic magnesium equivalent to 10 mg. per cent had little or no effect on the plasma magnesium in some calves, but caused a marked

improvement in others. An increase in the level of magnesium in the diet to about 34 mg. per cent led to a marked recovery in the plasma magnesium levels of grossly hypomagnesemic calves, but an increase to about 22 mg. per cent did not. Daily additions of 7000 I.U. of vitamin D to the diet before onset of hypomagnesemia did not prevent the subsequent appearance of this condition. Later increases in the intake of vitamin D by these animals, and addition of vitamin D to the diets of calves which became hypomagnesemic before receiving supplementary vitamin D, also was without appreciable effect.

For the first few weeks after birth the plasma calcium levels of all calves remained relatively constant but they subsequently developed hypocalcemia. It was found that in calves which developed hypomagnesemia before vitamin D was added to the diet, the decreases in plasma magnesium and calcium, respectively, began at about the same time and developed at about the same rate for any one calf. In all calves this relationship was destroyed when supplementary vitamin D was added to the diet. Complete restoration of normal plasma calcium took place within a month. However, the amount of vitamin D required to restore and maintain normal calcium retention appeared in some cases to be quite high.

Changes in the plasma magnesium and calcium levels did not appear to be associated with changes in the plasma inorganic phosphorus level or in the proportions of plasma magnesium and calcium which were ultrafilterable.

Growth of the calves appeared to be unaffected during early stages of magnesium deficiency. It was, however, sometimes inhibited after long periods of gross hypomagnesemia. Some calves suffered convulsions during the period in which their plasma calcium was reduced to about 50 per cent of normal. Although the plasma magnesium was below normal in these cases such depressions in magnesium alone were never associated with convulsions. This observation, plus the finding that vitamin D immediately stopped the convulsions, led the author to the conclusion that the convulsions were due to hypocalcemia rather than hypomagnesemia.

According to calculations made by the author, it appeared that the rate at which a calf developed hypomagnesemia was determined partially by the magnesium content of the diet but partially also by the rate at which the decrease in magnesium utilization occurred. As a consequence of the decrease in magnesium utilization, the minimum dietary requirements were concluded to increase greatly with age.

The decrease in magnesium utilization as the milk-fed calves became older could have been induced by decreases in absorption or an increase in endogenous fecal excretion, or both. The author is of the opinion that the changes were due to an abnormal condition arising from the particular properties of the milk diet, but he is unable to state what these conditions might be. The apparent correlation observed in the absence of supplementary vitamin D, between changes in plasma calcium and magnesium levels and retention, suggests the possibility of a related cause for the calcium and magnesium upsets.

## VITAMIN E AND SELENIUM, PART II

Separate lines of study by different groups have established a hitherto unknown mutual sparing effect between vitamin E and selenium (for part I of this review see *Nutrition Reviews* **16,** *149 (1958)*).

The groups of Schwarz and Scott have jointly published an article simultaneous with that of Patterson's group, entitled "Prevention of exudative diathesis in chicks by factor 3 and selenium" (K. Schwarz, J.

G. Bieri, G. M. Briggs and M. L. Scott, *Proc. Soc. Exp. Biol. Med.* **95,** *621 (1957)*). Scott's group, in the 1955 paper, had developed their vitamin E-free chick diet from Schwarz's Torula yeast ration known to be free from "factor 3", and had inferred that a factor in brewers' yeast protective against exudative diathesis might be Schwarz's factor. When Schwarz and Foltz showed that the latter was replaceable by selenite, the two groups studied the protective effect of selenium on exudative diathesis in chicks.

The authors used various "factor 3" preparations obtained during their purification studies and assayed them for protection against dietary liver necrosis in the rat. Selenium was tested as selenocystathionine, as sodium selenite and as elementary selenium. Chicks were started at one day. "Factor 3" activity in the rat correlated fairly well with protective activity against the chick exudative diathesis. Brewers' yeast or vitamin-free casein prevented the condition, as they prevent dietary liver necrosis. Selenocystathionine was protective against the rat lesion at a level equivalent to 4μg. per 100 g. of diet; 10μg. of selenium was protective against the diathesis in the chick either as selenocystathionine or sodium selenite. Elemental selenium was only effective in much higher doses. The growth of the deficient chicks was greatly impaired.

Schwarz *et al.* point out that selenium, until identified with "factor 3", has not been known to be beneficial to any animal species. They conclude, perhaps overcautiously, that selenium "may be a hitherto unrecognized essential trace element." The amounts involved are of the same order as those found in normal diets. Selenium as selenite or selenocystathionine proved 500 to 1000 times as active as tocopherol against the chick lesion, and 500 times in the rat. It is now clear that vitamin-free casein contains enough selenium to protect against vitamin E deficiency in both species. The authors believe, on the basis of unpublished work, "that the two substances act independently of each other in alternate pathways of intermediary metabolism."

Although a metabolic function has not previously been ascribed to selenium, the latter has been of biologic interest since its identification in 1933 in cereals which had been found toxic as a result of studies on "alkali disease" and "blind staggers" of livestock, now recognized as chronic selenium poisoning syndromes. This element is the subject of an excellent and comprehensive monograph by S. F. Trelease and O. A. Beath (*Selenium. Its Geological Occurrence and its Biological Effects in Relation to Botany, Chemistry, Agriculture, Nutrition and Medicine*. Published by the Authors. New York, 1949). Selenium poisoning has been extensively reviewed by A. L. Moxon and M. Rhian (*Physiol. Rev.* **23,** *305 (1943)*). The element is toxic for many animal species and is a problem in livestock and poultry raising. Symptoms of chronic poisoning appear in experimental rats when the diet contains 5 to 15 parts of selenium per million of diet and subacute symptoms appear at 15 to 25 p.p.m. Increasing the level of selenium progressively from 5 to 10 p.p.m. of diet reduces the hatchability of chick eggs progressively to zero. Chick growth is suppressed at the latter level.

It is interesting to note that rats fed small enough quantities of selenium to show chronic poisoning develop a severe anemia; "the most outstanding pathology is in the liver which is atrophic, necrotic, cirrhotic and hemorrhagic in varying degrees" (Moxon and Rhian, *loc. cit.*). This description refers to work published in 1940. One is left wondering about an element that can be at one time cause, and at another prevention of, the same lesion. Again, these observations need reinterpretation in the light of our present information about selenium and the other factors in dietary necrosis of the liver.

Although selenium has been generally thought of by workers in the medical sciences as a toxin, it is not quite true that no biological function has ever been imputed to

this element. It is essential for a number of plant species whose presence in seleniferous soils is so characteristic that they are considered "indicator" plants. Not only does their presence warn of a grazing hazard for livestock, but they concentrate selenium to to a level of several thousand p.p.m., compared to levels of usually less than 100 p.p.m. in cereals. Selenium accumulation in crop plants is correlated with their sulfur requirement and sulfate inhibits selenate uptake of these plants. However, the "indicator plants" do not have a high sulfur requirement, and sulfate does not inhibit their selenate uptake (Trelease and Beath, *loc. cit.*).

The authors of both of the papers on selenium and exudative diathesis could find only one clear demonstration that selenium has a biological function. This is the report of J. Pinsent (*Biochem. J.* **57,** *10 (1954)*) who found selenite (equivalent to 3 to 8 millimicrograms of selenium per ml.) essential for the formation of formic dehydrogenase by *Escherichia coli*.

It is tempting to speculate on the role of selenium in the organism. The amounts needed are comparable to those of some essential metals required because of their incorporation into metalloenzymes, rather than as structural elements. The apparent interchangeability of selenium, vitamin E and cystine, in order of increasing amount, suggests the possibility that selenium is part of an enzyme essential for some oxidation-reduction reaction involving cystine, a reaction in which vitamin E plays some part as an antioxidant.

Toxic selenium-containing cereals were early shown to contain the element as a part of the protein molecule, and the possibility that selenium might act by replacing the chemically similar element sulfur has been explored (Trelease and Beath; Moxon and Rhian, *loc. cit.*). The selenium analogue of cystine has attracted attention because its toxicity is greater than that of other selenium-containing organic compounds and is comparable to that of the element in grains and in sodium selenite.

Selenite can oxidize sulfhydryl compounds to form unstable —S—Se—S— compounds which are converted to —S—S— plus Se. Reduced glutathione is protective against toxic doses of selenite, as is arsenic.

Selenite has been known for years to inhibit certain enzyme systems (*e.g.* alcoholic fermentation) and sodium sulfide is partially protective. Selenium compounds have been shown to inhibit the action of succinoxidase and a variety of hydrogenases. Its inhibition of urease has been interpreted as further evidence of a relation between selenium and enzymes "dependent on the action of sulfhydryl groups." The relationships between sulfur, methionine and selenium in *Chlorella* have been reviewed (*Nutrition Reviews* **12,** *339 (1954)*).

One wonders whether vitamin E is entirely replaceable by selenium in the prevention of dietary liver necrosis. If the vitamin is not really needed here, is it anywhere? The other major lesions of vitamin E deficiency in brain, skeletal muscle, seminiferous epithelium, the fetal resorption, the deposition of ceroid pigment, can all now be re-evaluated in terms of the selenium content of the diet. The microcytic anemia of the vitamin E-deficient chick may be added to this list. It is possible that we may end with a quite new conception of the function of this vitamin in the organism.

In summary, parallel studies have been reviewed, showing that dietary liver necrosis in rats on a Torula yeast ration is prevented by cystine, vitamin E and "factor 3" of Schwarz, and that a similar factor is protective against the exudative diathesis of chicks on a vitamin E-free diet. The identification of selenium as an integral part of this factor led to the demonstration that sodium selenite is entirely protective against dietary liver necrosis in rats and the exudative diathesis of chicks and appears

to be an essential trace nutrient element. Finally, the implications of this as to the role of selenium in biological oxidation-reduction reactions are reviewed in the light of the element's known toxic and biologic functions, with the implications in regard to the function and essentiality of vitamin E.

## ALDOSTERONE AND STEROID GLYCOSURIA IN THE CAT

Diabetes has been produced in rats by the administration of cortisone but such a steroid is practically ineffective in producing glycosuria and diabetes in the cat or dog (W. A. Adelove and K. E. Paschkis, *Endocrinology* **55,** *637 (1954)*). Recently J. Buse, K. Gundersen and F. D. W. Lukens (*Diabetes* **6,** *428 (1957)*) found that when they gave 5 or 10 mg. of a microsuspension of 9α-fluorohydrocortisone per day subcutaneously to cats, hyperglycemia and glycosuria were produced in a series of seven of the ten cats studied. The doses of steroid used by these investigators are enormous if one considers that a human subject with adrenocortical insufficiency can be maintained on 0.2 mg. of 9α-fluorohydrocortisone. The glycosuria appeared between the fifth and twenty-seventh day of treatment. It first occurred on or before the seventh day in five of the cats. After the glycosuria had been established for three to four weeks, the 9α-fluorohydrocortisone dosage could be reduced to a level of 5 mg. per day and the glycosuria still persisted. At this time the blood sugar levels usually ranged between 128 and 191 mg. per 100 ml. After stopping the injections of the hormone, the glycosuria ceased within one to five days.

The appetites of the cats increased greatly within a day or two after receiving the massive doses of this hormone. Two of the cats developed a diuresis which usually does not accompany the intake of 9α-fluorohydrocortisone when given at low levels, but which has been noted by others in dogs which received a dose comparable to that given the cat. None of the animals developed visible evidence of edema, even though they received the hormone for as long as 89 days. However, no mention was made of their salt intake. Whether it was small enough so that sodium and fluid were not retained in abnormal amounts cannot be determined from the data presented.

After two or three weeks of receiving the hormone, at either dose level, the animals developed large abdomens. At autopsy or during an operation where biopsies of the pancreas were obtained, the cats were found to have enormous amounts of abdominal fat. The subcutaneous fat layers were of average thickness but the abdominal, omental, and perirenal fat depots were greatly expanded. The legs of the animals became thin and seemed slightly weak, but in general the cats behaved normally.

The skin became so thin that it offered no resistance to the needle used for the injection of the steroid. In four of the cats, there was atrophy of the adrenals which had also been noted previously by others following the administration of various adrenal steroids. These cats were also very susceptible to infection, and three of the animals died of diarrhea and pneumonia.

As glycosuria developed the animals were first excreting 1 to 2 g. of glucose per day with little or no elevation of fasting blood sugar. Gradually the glycosuria increased and the ability of the kidney to excrete sugar fast enough was exceeded, even though the sugar threshold of the kidney was reduced by the steroid administration. At this time the fasting blood sugar levels became elevated and were in the neighborhood of 200 mg. per 100 ml. After 89 days of giving the steroid, and almost three months of

severe glycosuria and hyperglycemia, sections of the pancreas showed severe hydropic degeneration of the $\beta$ cells.

After steroid administration was stopped, the animals ceased eating for up to five days. Six weeks after stopping the steroid and being on a reduced food intake, the glycosuria disappeared and the biopsy of the pancreas at this time showed that the hydropic degeneration had disappeared and the islet cells appeared normal. Thus there was development of the hydropic degeneration of the islet cells during glycosuria and subsequently complete recovery, both anatomically and functionally, after the administration of the hormone was stopped. There are previous reports, using the cat, where partial pancreatectomy, growth hormone administration or injections of massive amounts of glucose have been noted to cause similar lesions, and they too would disappear when treated early enough with either insulin, diet or phlorhizin.

In studying the effect of numerous irregular courses of steroid therapy on the glycosuria of cats, two animals received repeated bouts of steroid injections for long periods of time. These were interspersed with rest periods. One cat had 250 days of steroid therapy and 335 days of rest during a 22 month period. These investigators were unable to produce glycosuria in cats by giving 5 mg. per day of hydrocortisone for nine days or 50 mg. of hydrocortisone per day for a similar period. On the other hand, prednisone at a dose level of 50 mg. per day caused a glycosuria. Following repeated bouts of steroid administration and rest periods it was noted that the giving of 1.2 mg. of 9$\alpha$-fluorohydrocortisone would cause glycosuria. This dose was 12 to 24 per cent of that needed to cause glycosuria originally. Thus, the repeated bouts of steroid administration reduced the glucose tolerance of the cats.

The large increase in fat depots and the wasting of muscle, suggested that there is an accelerated protein break-down associated with a conversion of protein to fat or a sparing of fat. At autopsy the pancreas of the animals which had been maintained on varied hormone injections with interspersed rest periods were normal, except for well healed areas of scar tissue from the previous biopsies. In addition, the weight of the pancreas was normal. In one animal the adrenal weight was decreased following this series of hormone treatments, and in the other animal the adrenals weighed 149 mg. in contrast to an average weight of 586 mg. which these investigators noted for fifteen normal cats.

Thus it appears that prolonged steroid administration at these high dose levels has left some residual impairment of carbohydrate metabolism which lasted from three to four months after the steroid treatments were stopped. This was noted by slightly decreased glucose tolerance curves or the development of glycosuria when smaller doses of 9$\alpha$-fluorohydrocortisone were given. It is difficult to determine the physiological significance of these studies in view of the extremely large doses of hormone that were employed, but the development of hyperglycemia, the development of glycosuria, the influence on appetite, the production of obesity and the redistribution of body fat all are changes worth noting.

## VITAMIN $B_6$ AND TRANS-SULFURATION

The multiplicity of enzyme systems in which pyridoxal phosphate functions as coenzyme has resulted in some uncertainty as to its mechanism of action. Its functions in transamination and decarboxylation are at least understandable, but just what role it may play in tryptophan metabolism or in fatty acid transformation has remained obscure (*Nutrition Reviews* **4**, *232 (1946)*; **12**, *186 (1954)*; *Present Knowledge in Nutri-*

*tion, Second Edition, 1956*, The Nutrition Foundation, New York). In one field, sulfur transfer, the facts are now emerging rapidly and it is to be hoped they will lead to an understanding of how pyridoxal acts in these reactions.

F. Binkley (*J. Biol. Chem.* **194,** *109 (1952)*) reported that in livers of pyridoxine-deficient animals both the synthesis of cystathionine from homocysteine and serine and its cleavage to cysteine and homoserine are decreased. It was also shown that the two reactions are carried out by two separate enzymes (F. Binkley, G. M. Christensen and W. N. Jensen, *Ibid.* **191,** *531 (1951)*).

More recently H. Blaschko, S. P. Datta and H. Harris (*Brit. J. Nutrition* **7,** *364 (1954)*) investigated the urinary amino acids of rats on a pyridoxine-deficient diet. Among the known spots on the paper chromatogram was one which was tentatively identified as ethanolamine phosphate. In order to investigate the unknown spot more closely, D. B. Hope (*Biochem. J.* **66,** *486 (1957)*) raised rats of two strains, hooded Lister and Wistar, on pyridoxine-free diets. Urine was analyzed on a 2-dimensional paper chromatogram. The new spot appeared only in deficient animals of the hooded Lister strain (within about two weeks in males and four to five weeks in females). Since the spot in question was found actually to appear in the position previously assigned to cystathionine (C. E. Dent, *Ibid.* **43,** *169 (1948)*), the author proceeded to prepare enough of the material for a thorough identification. Elemental analyses, physical and chemical properties and known derivatives served to identify it as cystathionine, while X-ray diffraction studies and microbiological tests fixed the structure as that of the L(+) cystathionine, which promotes growth of young rats in the absence of cystine but not in the absence of methionine (W. P. Anslow, Jr., S. Simmonds and V. du Vigneaud, *J. Biol. Chem.* **166,** *35 (1946)*).

Two especially noteworthy facts emerged from these experiments. First, only one of the two deficient strains actually produced cystathionine in identifiable quantities in the urine. Second, although pyridoxine is required both for formation and splitting of cystathionine, this compound was formed in sizeable quantities in animals in which the cleavage enzyme apparently could not operate. It is possible that the enzyme responsible for formation of cystathionine has a greater affinity for pyridoxal phosphate than does that responsible for its breakdown. Again, the strain difference might be explained on this same basis, since in the deficient Wistar rats, it is possible that cystathionine was simply not formed.

## DIET AND THYROID FUNCTION

Contributions to our knowledge of the influence of diet on thyroid function have been frequently reviewed in these pages. The possibility that special therapeutic diets might lead to iodine deficiency in this country, where general iodine deficiency is infrequent at the present time, was suggested by the work of G. A. Bishopric, N. H. Garrett and W. M. Nicholson (*J. Clin. Endocrinol. Metab.* **15,** *592 (1955)*; *Nutrition Reviews* **13,** *327 (1955)*). In a group of hypertensive patients eating the "rice diet," which is low in protein and salt, half of the patients were found to have radioactive iodine uptakes which were elevated to the range usually associated with hyperthyroidism. The iodine content of these diets varies between 8 and 25 micrograms daily.

Another condition in which iodine deficiency may occur with some frequency in this country is in dietary cirrhosis of the liver (R. Mueller *et al. J. Clin. Endocrinol. Metab.* **14,** *1287 (1954)*). Nearly half of the 49 cases studied exhibited 24 hour thyroid

uptakes in excess of 50 per cent in the absence of clinical signs of hypermetabolism.

A more complete study of this problem has appeared recently. R. A. Shipley and E. B. Chudzik (J. *Clin. Endocrinol. & Metab.* **17,** *1229 (1957)*) have made observations on 36 patients. Thirty-three of these patients had alcoholic cirrhosis and none showed clinical evidence of thyroid abnormality. Five of the patients studied had 24 hour thyroid uptakes in excess of 46 per cent, which is the upper limit of the control group. Abnormalities in the metabolism of radioactive iodine were demonstrated in other ways. The ability of the thyroid to remove $I^{131}$ from the blood (thyroid clearance) was increased in seven patients. The degree to which inorganic $I^{131}$ in the plasma was converted to organic $I^{131}$ was found to be elevated in five patients at 24 hours. The percentage of $I^{131}$ present in the protein bound form was increased in only four of the subjects. In general, the patients demonstrating an abnormality in one test were abnormal in the other tests.

The results of measurements of nonradioactive iodine metabolism are somewhat equivocal. Three of the group of eight cirrhotic patients with abnormal $I^{131}$ tests had high $I^{127}$ uptakes in the thyroid, and high $I^{127}$ excretions in the urine. The other patients had low or normal measurements of these indices. All patients had normal values for the protein bound iodine.

In interpreting their results the authors point out that accelerated thyroidal uptake dissociated from augmented hormone secretion is a classic finding in dietary iodine deficiency. Although nutritional evidence is lacking that the diet in the eight patients with markedly abnormal $I^{131}$ metabolism was iodine poor, this may be suspected. The fact that these abnormalities disappeared after one to two months' ingestion of hospital food containing iodized salt strongly supports this contention. In view of these findings, the absence of goiter in the iodine deficient group is a puzzle. It is possible that the deficiency was of only mild severity and of insufficient duration to produce goiter.

Another mechanism whereby iodine deficiency may be induced has been described by L. Van Middlesworth (*Endocrinol.* **61,** *570 (1957)*). The author's curiosity was aroused by older reports of goiter and increased iodide requirement of animals kept under "unsanitary" conditions or fed soybean meal. The usual supposition that the goitrogenic effects of such diets were attributable to a dietary antithyroid substance was made unlikely by the observation that the uptake of $I^{131}$ was increased under such conditions. For this reason a more complete study of the effect of different diets on thyroid uptake and fecal and urinary excretion of $I^{131}$ was undertaken. A 26 per cent casein diet containing 10 per cent cottonseed oil was supplemented to the extent of 60 per cent with several types of crude and refined carbohydrate sources. As compared to gluten-sucrose, sucrose, starch and wheat, the largest thyroids were found in rats ingesting a soybean meal diet for two weeks. The starch and wheat diets had some goitrogenic effects, although none of the glands was as large as occurs with potent antithyroid agents. After three months on the soy diet the radioactive iodine uptake in the thyroid had risen to 84 per cent in six hours.

The route of excess iodine loss from the body on the soy diet was established by measuring the fecal radioactivity for three days after the intraperitoneal injection of $I^{131}$-labeled thyroxine. Almost twice the amount of radioactive iodine was recovered in the stools of the rats eating the soy diet. The lack of specificity of the soy diet was shown by the even greater loss of fecal $I^{131}$ when 30 per cent purified cellulose was incorporated into the diet. Of interest to many laboratory workers is the fact that a standard laboratory chow induced the greatest losses of fecal $I^{131}$. Examination of the plasma protein bound $I^{131}$ in the chow fed rats gave

values much lower than normal. The rats fed the soybean meal had intermediate values. A similar pattern in carcass $I^{131}$ was also observed.

The iodine in the feces is largely in the form of thyroxine and thyroxine glucuronides with possibly other degradative products of these compounds in which the iodine is still organically bound. The relatively small normal fecal iodine excretion in the rat is a small fraction of the organic iodine entering the gut by the bile. As A. Albert and F. R. Keating, Jr., (*Endocrinol.* **51,** *427 (1952)*) have shown, 100 per cent of the circulating thyroxine enters the gut per hour. Most of this thyroxine is reabsorbed to complete the entero-hepatic circulatory cycle. There are several possible ways in which soy flour, cellulose or laboratory chow augment fecal excretion of organic iodine. The increased bulk of these diets, by increasing the rate at which intestinal contents are propelled, might inhibit reabsorption. Alterations in the secretion of bile or the ability to reabsorb organic iodine might occur. It is also possible that on the starch diets there is more opportunity for degradation of thyroxine to iodide whose absorption would be more readily achieved. In any case, the goitrogenic effects of the high bulk diet should be preventable by increased iodide administration.

It is doubtful that a similar effect of dietary bulk will be demonstrable in man. The entero-hepatic circulation of thyroxine in man is very sluggish as compared to the rat. Direct measurements by N. B. Myant of the rate of excretion of biliary organic iodine have been reviewed (*Nutrition Reviews* **15,** *69 (1957)*). The "clearance" amounted to somewhat less than 35 ml. per hour which is much smaller than that which occurs in the rat. With the slight excretion of biliary thyroxine and metabolites it is unlikely that any alteration in dietary content could induce significant fecal losses.

## MINERAL BALANCE IN GUINEA PIGS

The guinea pig is highly susceptible to soft-tissue calcification, a condition which has also been referred to as the stiffness syndrome. Workers at the Missouri Agricultural Experiment Station, Columbia, noted this condition when guinea pigs were fed rations containing liberal amounts of phosphorus (A. G. Hogan, W. O. Regan, and W. B. House, *J. Nutrition* **41,** *203 (1950)*; *Mo. Agr. Exp. Sta. Bull.* **567** *(1950)*). From these studies it was apparent that guinea pigs were very sensitive to high levels of dietary phosphorus.

These workers further noted that the soft-tissue calcification in the guinea pig could be ameliorated with dietary supplements of magnesium and potassium (House and Hogan, *J. Nutrition* **55,** *507 (1955)*). These results suggested that guinea pigs have higher requirements for potassium and magnesium than the rat or the chick, an observation that was also evident from earlier work at the Wisconsin Station (P. Roine, A. N. Booth, C. A. Elvehjem and E. B. Hart, *Proc. Soc. Exp. Biol. Med.* **71,** *90 (1949)*).

It is of some interest that a deficiency of magnesium also has been implicated in metastatic calcification in other species, namely the calf (L. A. Moore, E. T. Hollman and L. B. Sholl, *Arch. Path.* **26,** *820 (1938)*), the cotton rat (M. A. Constant and P. H. Phillips, *J. Nutrition* **52,** *165 (1954)*), and the white rat (E. V. Tufts and D. M. Greenberg, *J. Biol. Chem.* **122,** *693 (1938)*).

Extending their investigations, the Missouri workers found that the high cation requirement of guinea pigs was caused in part by their inability to conserve fixed bases through the excretion of ammonia via the urine (B. L. O'Dell, J. M. Vandepopuliere, E. R. Morris, and A. G. Hogan, *Proc. Soc.*

*Exp. Biol. Med.* **91,** *220 (1956)*). On a basal ration containing 0.9 per cent of calcium, 0.4 per cent of phosphorus, 0.4 per cent of potassium, and 0.1 per cent of magnesium (levels which are widely used for rats and chicks), the guinea pigs grew at a subnormal rate. Increasing the potassium and magnesium to 1.5 and 0.4 per cent, respectively, was growth stimulatory. Increasing the phosphorus in the basal ration to 0.9 per cent severely suppressed the growth rate, increased blood phosphorus, and lowered the pH of the urine. When the dietary phosphorus was increased to 1.7 per cent by the addition of sodium dihydrogen phosphate, these effects were exaggerated, and the carbon dioxide-carrying capacity of the plasma was decreased from the normal level of 50 volumes per cent to 34 volumes per cent. Such a high phosphorus ration was obviously an acid diet, based on its calculated acid-base balance and on its effect on urinary pH and plasma carbon dioxide-carrying capacity.

The effects of the high-phosphorus ration could be overcome largely by increasing the dietary levels of potassium and magnesium to 1.5 and 0.4 per cent, respectively.

The urinary pH appeared to be very sensitive to slight changes in the acid-base balance of the ration. It served as a criterion in studies designed to evaluate various cations in counteracting the injurious effects of the high-phosphorus ration. Supplementary sodium, potassium, magnesium and calcium were all effective in improving growth and in raising the urinary pH.

Measurement of the urinary ammonia revealed that even on acid (high-phosphorus) rations, the guinea pigs excreted only very small amounts of ammonia nitrogen (1.1 mg. per 100 ml.). This is to be compared with ammonia concentration in human urine of about 60 mg. per 100 ml. Thus, consumption of an acid diet by the guinea pig caused a change from basic to acidic phosphates in the urine, but did not stimulate the excretion of urinary ammonia. This shift to acid urine appears to represent a mechanism for conserving fixed bases under an acid-diet situation. The Missouri workers suggested that the high blood phosphorus and the soft-tissue calcification, observed when guinea pigs consume the high-phosphorus diets, is due in part to the inability of the guinea pig to excrete phosphorus normally under a condition of low-base reserve (relatively low dietary potassium and magnesium).

In a more recent study these investigators have measured cation intake and output in adult guinea pigs (O'Dell, Morris, E. E. Pickett and Hogan, *J. Nutrition* **63,** *65 (1957)*). Balances (ten-day) were determined for calcium, magnesium, potassium, sodium, and phosphorus under essentially the same dietary conditions as were used in the previous study.

The apparent absorption of calcium and magnesium was decreased about 50 per cent under conditions of high-phosphorus intake. When the diet contained 0.4 per cent of phosphorus, the apparent calcium and magnesium absorptions were 70 and 84 per cent, respectively, contrasted with apparent absorptions of 37 and 46 per cent, respectively, when the diet contained 1.8 per cent of phosphorus. The apparent absorption of sodium was not influenced by the dietary phosphorus level. There was a trend toward lower absorption of potassium as the dietary phosphorus level was increased. The balance data established that the high-phosphorus diet consistently resulted in negative magnesium and potassium balance.

From these results it is apparent that the guinea pig is unique in that alimentary calcium and magnesium absorption is higher than in most other animals studied, and that the bulk of these absorbed minerals is excreted via the urine.

The Missouri workers feel that because of the inability of the guinea pig to conserve fixed bases, and its failure to increase urinary ammonia excretion, a negative potassium balance results when high-phosphorus rations are fed.

Since a magnesium deficiency is known to

cause calcinosis in other species, the soft-tissue calcification in the guinea pig is reasonably explained as a magnesium deficiency due to interference of its absorption by excess dietary phosphorus.

It has been recognized and confirmed by many investigations that a high-calcium diet suppresses magnesium absorption. Surprisingly, the detrimental effect of phosphorus on the absorption of magnesium, as reported by the Missouri researchers, appears not to have been recognized previously.

These careful investigations again point strongly toward the need for recognizing the importance of proper balance in dietary minerals. The results also emphasize that dietary mineral needs vary strikingly with animal species.

## FASTING AND IN VITRO LIVER PROTEIN RELEASE

It is recognized that, in the absence of food, the body secures a large share of its energy from fat and almost immediately begins to lose nitrogen. The work of T. Addis, L. J. Poo and W. Lew (*Nutrition Reviews* **9,** *103 (1951)*) showed that during a two-day fast, the liver was the only organ which lost any significant amount of nitrogen; the heart, kidney and the other organs lost an insignificant amount.

An interesting suggestion of an *in vitro* demonstration of the loss of protein from the livers of fasted rats has been reported by E. Kaufmann and E. Wertheimer (*Am. J. Physiol.* **190,** *133 (1957)*). In studying nitrogen metabolism in rat tissues these investigators, at the Hebrew University in Jerusalem, noticed that liver slices of fasted rats consistently released more nitrogen into the buffered medium than did those of fed rats. In an extension of this work, they fasted male albino rats weighing 100 to 150 g. for a period of 18 hours. At the end of that time, the rats were killed. Tissue slices 0.5 mm. thick were cut and placed in a Krebs-Ringer phosphate solution. The amount of nitrogen in the medium at the end of one-half hour of incubation was subtracted from that present after three and one-half hours of incubation. The difference between these two values expressed in terms of 100 mg. of tissue was the basis for their calculations. The initial half-hour value for nitrogen loss, according to them, represents physical diffusion due to tissue injury (*see* D. L. Kline, *Endocrinology* **45,** *596 (1949)*).

The buffer medium containing the livers of the rats that had been fasted for 18 hours contained 225 micrograms of nitrogen for each 100 mg. of liver tissue. Livers from rats that had been fed until killed lost only 100 micrograms of nitrogen to the buffer medium under the same conditions. Both sets of liver slices lost the same amount of non-protein nitrogen (80 micrograms). When the total nitrogen values are corrected for non-protein nitrogen, the difference between the liver slices from the fasted and fed rats becomes even greater (20 vs. 145 micrograms of nitrogen). The investigators infer that the material liberated from the liver slices is protein, but they provide no proof for their statement.

When diaphragm, kidney and spleen slices from fed and 18-hour fasted rats were tested under the above conditions, the same amount of nitrogen was liberated by the tissue from the two groups of animals. This observation agrees with the work of Addis, Poo and Lew (*Ibid.*) who found that the liver was the primary site of protein loss early in starvation.

In order to determine whether the above effect was non-specific, Kaufmann and Wertheimer (*Ibid.*) analyzed the buffer medium for ribonucleic acid. The liver slices from the fasted rats released 76 micrograms of ribonucleic acid after three hours of incubation, while the liver slices from fed rats released

68 micrograms. This difference in ribonucleic acid release between the fasted and fed rats was insignificant. Since the difference for the protein nitrogen was highly significant, the investigators felt the phenomenon was related to the physiological condition of the animal.

Fasting for nine hours resulted in a difference in nitrogen liberation by the liver slices of the fed and fasted rats which was approximately half that observed with the rats that had been fasted 18 hours. Prolonging the fast to five days showed no further increase in nitrogen release over that of the 18-hour fasted rats.

The "fasting effect" was the term given by the investigators to the difference in nitrogen release by the livers of the fasted and fed rats. After a five-day fast, it required one day of re-feeding to abolish the "fasting effect." After two days of re-feeding, the nitrogen released by the livers of the re-fed rats was less than that of the continuously fed rats; after four days of refeeding, the nitrogen release by the liver slices was again the same for the two groups.

Suggestive evidence that the caloric intake plays an important role in regulating the release of nitrogen from the liver slices was obtained from the following experiments. When a five-day fast was followed by refeeding a protein-free diet for one night, the nitrogen release returned to normal just as it did when the fasted rats were re-fed the "standard" diet (composition not stated). However, if the overnight re-feeding was accomplished with only 40 per cent of the daily food intake (standard diet) of full-fed rats, there was no reduction in the nitrogen released from the livers.

The diet fed the rats just prior to the fasting period had an influence on the amount of nitrogen released by the liver slices. If a protein-free diet were fed for five or nine days (producing a 10 and 12 per cent loss in body weight, respectively), the liver slices from the rats killed after an eighteen-hour fast still showed the increased nitrogen liberation when compared with the liver slices from the fed rats. Underfeeding the rats at a level of 40 per cent of their regular intake (standard diet) slightly decreased the difference between the fasted and fed rats. Feeding the rats a high protein diet (70 per cent casein) for ten days prior to the 18-hour fast reduced still further the difference between the fed and the fasted animals. Feeding a high fat ration (69 per cent margarine) for ten days prior to the fast completely eliminated the difference between the fasted and fed rats.

Although the investigators did not attempt to explain the above observations, they do fit in with the relative magnitudes of the nitrogen loss that one would expect during a fast following the ingestion of the different diets.

The administration by stomach tube of 3 ml. of 40 per cent glucose solution to rats 18 hours after fasting produced a reduction in the protein released by the liver. Two hours after the administration of glucose the nitrogen released by the fasted rats was approximately the same as that of the livers of the control rats. The reduction in the nitrogen liberation by the livers of the fasted rats paralleled their increase in glycogen content. When arabinose was given to the fasted rats in amounts equivalent to the glucose, the nitrogen released by the livers of the fasted rats remained high.

When similar studies were carried out on rats ranging in age from two to 18 weeks, the protein liberated by the livers of the fed rats showed practically no change with age. The livers of the rats fasted for 18 hours, however, showed a progressive increase over their fed control animals, with a difference between the two groups, going from approximately 0 for the 30 g. rats to 260 micrograms of total nitrogen per 100 g. of tissue after three hours of incubation for the 200 g. rats.

These findings suggest that when liver slices are incubated in a solution of salts protein is released from the liver slices in amounts that depend upon the nutritional status of the animal. The authors suggest that the ability of the liver to retain its

protein was connected with carbohydrate metabolism and that the deposition of glycogen might form a protein-glycogen compound as was suggested by a number of workers (*see* A. Lazarow, *Anat. Rec.* **84,** *31 (1942)*).

Kaufmann and Wertheimer (*Ibid.*) suggest that an active metabolism of carbohydrate is necessary for the retention of protein by the liver slices. When insufficient carbohydrate is present to supply the energy requirements, some mechanism is called into play which enhances the depletion of liver protein. Once this process has been initiated, it manifests itself by an increased liberation of protein by the liver slices incubated in a buffer medium. The investigators point out that there are a number of observations which do not agree with their theory, but it appears to be the best that can currently be offered.

There are a number of questions that should be considered as guides for an extension of the above work:

1.) Are there any histological differences in the liver slices, after the incubation period, which might explain the differential loss of protein?

2.) If the cell structures are intact, then how can such large amounts of protein get through the cell walls? The amount of protein released represents approximately 10 per cent of that originally present in the liver.

3.) What is the composition of the livers in terms of protein, fat and moisture? Values such as these are essential in order to establish a correlation between the *in vitro* and *in vivo* effects.

4.) What is the nature of the protein that is being liberated and how is it related to the tissue that is lost by the intact animal during the fasting period?

The observations of Kaufmann and Wertheimer (*loc. cit.*) are interesting in suggesting an *in vitro* response that mirrors, to a certain extent, an *in vivo* reaction.

## THE ADRENAL CORTEX IN CARBOHYDRATE METABOLISM

The metabolism of carbohydrate, protein, and fat is profoundly influenced by hormones of the adrenal cortex. The nutritionist is directly concerned with the manner in which alterations in adrenal function may determine the fate of nutrients in the body. The general aspects of this problem were described in a masterly way by C. N. H. Long, B. Katzin and E. G. Fry (*Endocrinology* **26,** *309 (1940)*) who clearly defined the importance of the adrenal in gluconeogenesis in the fasting state. Despite continuous investigative effort by many workers during the ensuing years, the field today appears more complicated than ever, and there is an obvious lack of understanding of the fundamental action of adrenal cortical steroids.

A paper of interest in this field has appeared from the Yale group, (W. W. Wintenitz, R. Dinziz and C. N. H. Long, *Ibid.* **61,** *724 (1957)*), in which the changes in carbohydrate metabolism following epinephrine were analyzed. In the normal fasted rat, epinephrine precipitated a fall in muscle glycogen but a striking rise in liver glycogen, which, at four hours, was as much as 12 times the control level. In contrast, in the fasted adrenalectomized rats, epinephrine produced a somewhat greater percentile decrease in muscle glycogen, but no rise in liver glycogen. Blood lactate rose higher in the adrenalectomized animals after one hour, but promptly fell to normal levels after four hours. A rough overall carbohydrate balance was calculated by estimating total hepatic and muscle glycogen and the glucose and lactate in the extracellular space. A much greater amount of carbohydrate was unaccountable in the adrenalectomized rats than in the normal animals.

The failure of liver glycogen to rise after epinephrine in the fasted adrenalectomized

rat is not to be attributed to a failure of lactate production from muscle glycogen, nor to a failure of the liver to convert lactate into glucose. Actually the rise in blood glucose was as great in the adrenalectomized rats as in the intact rats. The defects cannot be corrected by desoxycorticosterone, but were reversed by cortisone pellets and adrenal cortical extracts.

The authors clearly localized the defect in the liver by performing experiments with eviscerated animals. The control animals had been subjected to adrenodemedullation to avoid epinephrine discharge during the evisceration. In these experiments the breakdown of muscle glycogen and the rise in blood lactate after epinephrine were not significantly different in the demedullated and adrenalectomized rats.

The effects of epinephrine in the diabetic animal were compared with those in the adrenalectomized animal after four hours. In each there was a marked decrease in muscle glycogen and failure of liver glycogen to increase. The diabetic animal exhibited an exaggerated blood sugar rise and glycosuria. The calculated carbohydrate balance, however, failed to show the large amount of unaccountable carbohydrate observed in the adrenalectomized animal.

Fasted adrenalectomized rats demonstrated a severe impairment in the ability to convert infused glucose, fructose, glycerol, lactate and malate into liver glycogen. This abnormality could be corrected by hydrocortisone administration.

These observations indicate that in the adrenalectomized rat less of the lactate mobilized from the muscle appears as liver glycogen and more disappears by other pathways. The nature of these alternate pathways was not defined. The authors were unable to find evidence of accelerated lipogenesis. Increased oxidation of lactate to carbon dioxide was indicated by the higher respiratory quotient found in adrenalectomized rats receiving epinephrine (Winternitz and D. L. Kline, *Fed. Proc.* **15,** *202 (1956)*). The authors also speculate that adrenal cortical steroids might control the proportion of glucose which is metabolized by way of the phospho-gluconate shunt, and in this way influence the metabolism of ribonucleic acid and nucleotides. Nothing in their present work pertains to such a suggestion.

Another puzzling feature of the action of adrenal cortical steroids is brought out by a paper of P. M. Hyde (*Endocrinology* **61,** *774 (1957)*). Liver glycogen and liver radioactivity were measured at intervals after the intravenous and intragastric administration of 3.0 mg./Kg of cortisol-4-$C^{14}$ to fasting rats. It was noted that radioactivity was nearly absent from the liver by the time that glycogen had significantly increased, and more than two hours before the maximal increase in liver glycogen had occurred. Although not conclusive, this type of observation suggests that the continued presence of cortisol is not required for sustained enzymatic alterations. Hyde suggests that cortisol functions as a trigger mechanism to increase the enzymatic machinery necessary for hepatic glycogen synthesis. This increase in enzymatic activity then persists for considerable periods after the steroid has left the tissue.

We still await an integrated picture of adrenal steroid action and there are many oddly shaped pieces to be fitted together. The common denominator between enhanced glycogenesis, gluconeogenesis, inhibition of protein synthesis, and increased lipogenesis has not been found.

## TOXICITY OF CHOLESTEROL-FATTY ACID MIXTURES

Several recent reports by J. Raulin (*Arch. Sci. Physiol.* **8,** *1, 107, 195 (1954)*); **10,** *1 (1956)*; *Proc. 2nd Intern. Conf. Biochem. Prob. Lipids. Ghent, 1955, p. 447*) have indi-

cated that a diet containing 16 per cent of unsaturated acids and 3 per cent cholesterol is toxic to weanling rats. The symptoms of toxicity resemble those of choline deficiency (multiple hemorrhage, fatty liver, early high mortality rate, etc.). These toxic symptoms are prevented if extra choline is included in the diet or administered by injection, or if the fatty acids are given as triglycerides. However, glycerol, betaine, inositol and tocopherol are ineffective in this respect.

In order to investigate the mechanism of the toxicity of this diet, G. Clement, J. Clement and J. Raulin (*Arch. Sci. Physiol.* **11,** *101 (1957)*) have studied the effects of the toxic diet, and four closely related diets, on the lipids of various tissues of rats. Weanling rats were placed on adequate diets identical with the following exceptions: diet A contained 16 per cent triglycerides of sunflower seed oil and 1 per cent choline chloride; diet B contained the choline and 15 per cent sunflower seed oil fatty acids; diet C contained the oil, 3 per cent cholesterol and 0.05 per cent choline; diet D (the toxic diet) was like C but contained the fatty acids; diet E was like D but contained 1 per cent choline. Analyses were carried out on the intestinal contents, feces, intestinal epithelium, blood (cells and plasma) and liver. The lipids assayed were the glycerides, phospholipids, free fatty acids, soaps, unsaponifiables, cholesterol and cholesterol esters. The iodine numbers of the fatty acids were also checked in most cases.

Consideration of the analytical results revealed few variations in the lipids of the intestinal epithelium ascribable to dietary changes. The blood picture, on the other hand, appeared too complex and variable for interpretation. Interpretable results were obtained, therefore, only for the liver, intestinal contents and feces.

In the case of the liver, the concentration of phospholipids was lowest on the toxic diet (D), and the iodine number of the phospholipid fatty acids was much lower on that diet than on any of the others. As was to be expected, triglycerides and sterol esters were present in greatest amounts when the diets contained little choline (C and D), showing development of fatty livers.

The lipids of the intestinal contents were evidently composed of those lipids which were not yet absorbed and those which had been excreted through the intestinal wall. No phospholipids were found on diets A, B or C, but large amounts appeared on both diets D and E. In this connection, it was noted that significantly more was found when the diet contained little choline (D) than when it contained extra choline (E). Cholesterol appeared in significant amounts in the intestinal contents only when it was present in the diet (C, D and E), but cholesterol esters did not appear to depend on dietary cholesterol, since they were present in similar concentration in all diets except the two which were low in choline (C and D), in which they were considerably lower.

The fecal analyses followed those of the intestinal contents in that the phospholipids were present in large amounts only in feces of rats on diets D and E.

From these results, the authors concluded that the dietary combination of fatty acids and cholesterol promoted the loss of phospholipids into the digestive tract and their elimination in the feces. This loss resulted in a choline deficiency in the case in which the diet contained little choline (D). When extra choline was added, however (diet E), the same loss took place but was alleviated by the increased intake. These findings agree with those of K. S. Kim and A. C. Ivy (*Am. J. Physiol.* **171,** *302 (1952)*) who reported that corn oil fatty acids fed with cholesterol promoted its absorption and produced higher serum cholesterol levels than did corn oil. These same authors found that corn oil fatty acids increased phospholipid excretion in the feces to 14 times that when the corn oil itself was fed.

Clements and Raulin consider that the increase in cholesterol esters present in the intestinal contents of animals on the diets containing extra choline is due to an increased formation *in situ*. Since cholesterol

esters are formed preferentially from the more highly unsaturated acids, when these are present in large excess, they might be converted to cholesterol esters which could be absorbed very rapidly and would result in lowering of the iodine number of the remaining free fatty acids, an occurrence which was confirmed by the experiments. That this is an abnormal occurrence is pointed out by the authors, who had previously reported that during hydrolysis of normal triglycerides by pancreatic lipase, the more saturated fatty acids are liberated first (G. Clement and J. Clement, *Bull. Soc. Chim. Biol.* **36,** *1319 (1954)*; **37,** *857 (1955)*; *Arch. Sci. Physiol.* **10,** *73 (1956)*).

Thus, the authors believe that the primary disturbance is in the lumen of the intestine and that the alteration in the character of the lipids therein results in deranged absorption and consequent loss of choline-containing phospholipids.

In view of the recent findings of H. Gordon, B. Lewis, L. Eales and J. F. Brock (*Nature* **180,** *923 (1957)*) that unsaturated fatty acids promote excretion of both cholesterol and bile acids, the products of cholesterol metabolism, another possibility can be considered. First, a greatly increased rate of cholesterol absorption is promoted by unsaturated fatty acids when the two substances are fed together. Second, the unsaturated fatty acids are responsible for aiding transport of cholesterol to the liver for excretion in the bile as sterol or bile acid. This function of the fatty acids could be performed as esters of cholesterol and as part of the lecithin molecule. Finally, the lecithin involved could be destroyed in the liver or excreted in the bile to be lost in the feces. In the case of animals on a low choline diet, such a process, taking place rapidly, could result in the symptoms of choline deficiency. Additional choline would alleviate the symptoms but would not reduce the loss of phospholipids in the feces.

These experiments serve as an illustration of the complex picture of fat absorption, transport and excretion which can be obtained when all three processes are studied at the same time. It is this type of study which will eventually lead to the elucidation of these problems which are closely related to the question of the effect of dietary fat in atherosclerosis and other diseases.

## NUTRITION STUDIES IN THE COLD

The influence of diet on the ability of animals to survive at low environmental temperatures has been studied extensively in recent years. Associated with an increase in energy requirement of animals exposed to cold is an increase in requirement for certain specific nutrients. These include ascorbic acid (L. P. Dugal and M. Therien, *Canad. J. Res.* **25 *E*,** *111 (1947)*) and pyridoxine, riboflavin, and vitamin A (B. H. Ershoff, *Proc. Soc. Exp. Biol. Med.* **78,** *719 (1951)*; **79,** *559*, *580 (1952)*).

In an extensive review of the relation of nutrition to climatic stress it was concluded that high protein intake was not beneficial in reference to cold tolerance, that a high carbohydrate diet was superior to one high in protein, and that high dietary fat under some conditions improved still further the benefits from starchy foods (H. H. Mitchell and M. Edman, *Nutrition and Climatic Stress, with Particular Reference to Man*, Charles C. Thomas Publ. Co., Springfield, Illinois (1951)).

A suggestion that cold environment influenced fat metabolism or utilization can be found in the data presented by E. A. Sellers and S. S. You (*Biochem. J.* **51,** *573 (1952)*). These workers found that when rats were exposed to cold the deposition of excess fat in the liver produced by feeding hypolipotropic diets was effectively prevented. Re-

cent studies have confirmed this observation and have revealed further an interesting relationship between environmental temperature and the efficiency with which protein is utilized (C. R. Treadwell, D. F. Flick and G. V. Vahouny, *J. Nutrition* **63,** *611 (1957)*).

These workers investigated the influence of dietary protein and fat level in purified diets fed to four-week-old and adult rats. Comparable groups of animals were fed the various diets at environmental temperatures of 1° C and 25° C.

In diets one to five the protein (casein) in the diet was varied between 5 per cent and 40 per cent at the expense of the carbohydrate components (starch and sucrose) with the fat constant at 20 per cent (18 per cent of lard and 2 per cent of cod liver oil). Diets six, three, seven and eight contained fat at levels of 10, 20, 30, and 40 per cent, respectively, at the expense of carbohydrate and with protein constant at 20 per cent. In addition to salt mixture (5 per cent) and celluflour (2 per cent) the diets contained a liberal fortification of all the vitamins known to be required by the rat, except that choline and inositol were not included. Thus, the only source of lipotropic factors in the diets was the methionine in the casein. In preliminary experiments both young (50 to 60 g.) and older (150 to 180 g.) rats died from frostbite at the temperature of 1° C when they were housed in metal cages with raised wire mesh bottoms. Satisfactory survival for the 28-day experiments was accomplished in the cold environment by covering the cage bottoms with perforated, plastic matting.

At the 5 and 10 per cent dietary protein levels the growth rate of 60 to 80 g. rats was distinctly better in the cold environment than at the 25° C environmental temperature. The rats kept at 1° C averaged about 2.5 g. in daily body weight gain, while those at 25° C averaged about 0.9 g. when the diet contained 10 per cent of protein. The rats receiving the diets containing 5 per cent of protein averaged 0.5 g. daily in weight gain at 1° C temperature, while those in the warmer environment actually exhibited a slight weight loss. Since caloric intakes were equal, it is evident that the cold environment increased the efficiency of protein utilization.

Contrasted with the low-protein diets, daily gains of 4 to 5 g. were attained by rats receiving 20 to 40 per cent of protein at 25° C temperature, while those in the cold (1° C) averaged about 3.0 g. on the higher protein diets. With the exception of the 5 per cent protein diet, the cold environment resulted in increased total food and caloric intakes.

When the protein content of the diet was held constant at 20 per cent and the fat content varied, the growth rate at 25° C was higher at the 10, 20, and 30 per cent fat levels and lower at the 40 per cent level than at 1° C. The protein efficiency in the 1° C environment gradually increased with increasing fat content, so that with the 40 per cent fat diet the two environmental temperatures exhibited about equal efficiencies. In the warmer temperature protein efficiency was maximum at the 20 per cent fat level and decreased at higher fat levels.

The liver fat levels were uniformly the same on all dietary protein and fat levels when the rats were kept in the cold environment. In contrast, fatty livers were present in all rats receiving diets containing 5, 10, and 20 per cent dietary protein levels, and in all rats receiving 20, 30, or 40 per cent dietary fat in the 25° C environment. These findings clearly reveal that cold environment is a very effective lipotropic agent.

It is obvious from the data that, using the level of liver fat as a criterion, the rat held in a cold environment has an increased capacity of metabolizing fat.

These studies are of particular interest since peoples in cold climates voluntarily ingest large quantities of fat and may exhibit "fat-hunger". The striking effect of cold on liver fat in animals on hypolipotropic diets suggests that lipid metabolism is controlled through mechanisms that are, as yet, poorly understood.

## SPERMINE AS A GROWTH STIMULANT

Many efforts have been made to identify those specific materials in enzymatic hydrolysates of protein which are "growth stimulants" for the multiplication of *Lactobacillus casei* and other bacteria. The effective ingredient of such digests was originally termed "strepogenin" by H. Sprince and D. W. Wooley (*J. Exp. Med.* **80,** *213 (1944)*) who believed it to be a single substance or closely related group of substances. Glutamine and a variety of peptides have been shown to be somewhat active as stimulants. However, H. Kihara and E. E. Snell (*Proc. Nat. Acad. Sci.* **43,** *867 (1957)*) have observed that none of these purified substances is as effective in promoting growth of *L. casei* as a commercially available pancreatic digest of casein. This fact led these investigators to suspect that stimulation was due to non-peptidic materials introduced with the cruder protein preparations used in preparing such commercial digests.

In an attempt to elucidate further these factors H. Kihara and E. E. Snell (*loc. cit.*) added ascorbic acid, adenylic acid, uracil and guanylic acid to a basal medium (H. Sprince and D. W. Wooley, *loc. cit.*), supplemented in the one case with a trypsin digest of purified casein and in another case with the crude pancreatic digest of casein. The added nutrients seemed to induce a substantially earlier and heavier growth of the test organism in both instances. However, the difference between the two preparations was not eliminated. All subsequent tests were conducted in the basal medium as supplemented by these vitamins.

It was found that acid hydrolyzed pancreatic digest was inactive by itself, but when purified casein digest was added with the hydrolyzates the growth responses closely approximated those of non-hydrolyzed pancreatic digests. This finding confirmed the supposition that the difference in the preparations was due mainly to non-peptidic substances (acid stable), and demonstrated that these unidentified substances were active only in the presence of appropriate peptides.

Further tests showed that the active substance was: (a) retained by the cation exchange resin, Dowex-50, but not by the anion exchange resin, Dowex-1; (b) adsorbed by charcoal at pH 7; (c) eluted from charcoal by acidic ethanol; and (d) distilled with steam from the charcoal eluate when this was made strongly alkaline. These properties are possessed by steam volatile amines, and specifically by spermine and related materials.

Tests of several amines indicated that spermine and spermidine were highly active; putrescine was less than 0.001 as active on a molar basis. Related compounds including cadaverine, agmatine and arcaine were inactive as were histamine, tyramine and tryptamine. Slight activity similar to that of putrescine was shown by 1-3 propanediamine; N-(3 aminopropyl)-1,3-propanediamine was nearly as active on a molar basis as spermidine, but less than spermine and did not permit such heavy growth. Each of the active compounds was toxic at concentrations substantially above that required for maximum growth.

Supplementation with spermine largely, but not completely, eliminated the difference in growth response to the two partial digests of casein. Like the acid-stable component of pancreatic digest, spermine did not stimulate growth in the absence of a peptide source.

Although E. J. Herbst and E. E. Snell (*J. Biol. Chem.* **176,** *989 (1948)*; **181,** *47 (1949)*) have shown spermine and spermidine to be essential growth factors for *Hemophilus parainfluenzae*, and other bacteria have likewise been shown to require these substances, their functions are still unknown. Their dependence upon intact peptides for activity is most curious. It is possible that these substances also enter into the metabolism of mammalian cells, a possibility which should be tested. However, the likelihood that this metabolic pathway is unique to bacteria (which grow in an environment especially rich in these factors) is quite great.

# NOTES

## Food Faddism

The leaflet "Food Facts vs. Food Fallacies" issued in April, 1957, by the Food and Drug Administration, is recommended to the attention of all who are interested in improved health. Copies of this statement are available by writing to the Food and Drug Administration, Department of Health, Education and Welfare, Washington 25, D. C. The following are excerpts from the text of the leaflet:

"The American food supply is unsurpassed in volume, variety, and nutritional value. By patronizing all departments of a modern food store we can easily supply all of our nutritional needs . . .

"Notwithstanding the abundance and quality of the American food supply a persistent campaign is being carried on to undermine public confidence in the nutritional value of staple foods. False ideas about food are circulated by food faddists and by fringe promoters of vitamin and mineral products. Such products are sometimes offered cure-alls for serious disease conditions. This may be dangerous to health, especially if ailing people are led to put off getting proper medical attention.

"Modern as well as ancient myths and superstitions about food are utilized by faddist promoters. Such notions as the old idea that fish and celery are 'brain foods', or that oysters increase fertility, are harmless, but when garlic pills are promoted for high blood pressure, or grapes for the treatment of ulcers and cancer, the price of ignorance may come high. There is quackery in the field of nutrition as well as in the field of medicine.

"Today the Food and Drug Administration is especially concerned about the promotion of 'food supplements' as cure-alls for conditions which require medical attention.

"Misleading promotion of food supplements violates Federal law. It is commonly carried on in two different ways:

(1) One method is by so-called 'health food lecturers' who claim, directly, or by inference, that the products they are promoting are of value in preventing or curing disease, when in fact they are ineffective for such purposes. Some of these lecturers put on a program that has the emotional appeal of a 'revival' meeting. Others pose as highly qualified scientists. Their talks are a blend of science and superstition concerning nutrition, right living, psychology, sex, and, of course, salesmanship for the products which the particular lecturer happens to be pushing. Usually an ad is placed in a local newspaper announcing several free lectures. These are the 'come-on' for the paid series which follows.

(2) A second and widespread method is by door-to-door selling. While this may be carried on as a legitimate business, there are, unfortunately, agents who take advantage of the privacy of the home to prescribe high-priced food supplements for any disease or condition which an ailing customer may mention.

"Both kinds of sales agents use a scare technique in selling their vitamin, mineral, and herbal preparations. False ideas about food are their stock in trade. . . . They can easily be debunked by making sure certain facts are not overlooked . . .

"Nutrition authorities agree that the best way to buy vitamins and minerals is in the packages provided by nature—vegetables, fruits, milk, eggs, meats, fish, and whole grain, or enriched bread and cereals."

## The Fluid Intake of Small Children

There is very little information in the literature concerning the fluid intake of preschool children, particularly that of drinking water *per se*. Since the water intake of chil-

dren in this age group is of quantitative interest to those interested in fluoridation as a measure to control dental caries, H. H. Neumann (*Arch. Pediat.* **74,** *465 (1957)*) has conducted a survey by questionnaire of the mothers of 312 children below the age of six years living in suburban Long Island. The children ranged in age from six months to six years, and there was little variation in the fluid intake over this age range. The fluids consumed by this group of children consisted primarily of milk, tap water, and tap water used to dilute frozen orange juice. The principal source of fluids was fresh cow's milk with an average consumption of 840 cc. per day. The consumption of the upper third was 1,005 ml. per day, and that of the lower third an average of 647 ml. per day, so that there was very little variation among the group as a whole.

The average tap water intake was 294 ml. per day. In this category the variation was considerable, the lowest third consuming an average of 85 ml. while the mean intake of the upper third was 618 ml. per day. Twenty-nine per cent of the mothers claimed that their small children hardly ever drank tap water. The only other fluid consumed habitually and regularly was orange juice, 66 per cent of the children drinking prepared frozen orange juice, 26 per cent fresh juice, and 8 per cent none. The average amount of tap water consumed with frozen orange juice was 99 ml. Other fruit juices were used only rarely. The consumption of carbonated beverages was low, only one child in 35 being allowed carbonated drinks daily.

The author expressed interest in the comparison of the milk and water drinking habits of this group of children with those in other geographic areas. Such data have recently appeared in the studies of V. A. Beal (*Nutrition Reviews* **16,** *67 (1958)*). Her data on milk consumption of Denver children show an average milk intake which is slightly lower at both six months and six years with a dip to 480 ml. per day at about 2½ years. Her more detailed study would serve to confirm at least the general range of the figures reported by Neumann as far as milk intake is concerned.

These data concerning the tap water intake of preschool children would certainly suggest that some members of this group may not be ingesting fluorides added to municipal water supplies in very large amounts, and also demonstrate the great variability in tap water intake in this age group. On the other hand, they are reassuring in demonstrating the prevalence of intakes of whole cow's milk which, according to Beal, are apparently optimal according to theoretical calculations.

### Carotenoid Stores in Man

D. H. Blankenhorn (*J. Biol. Chem.* **229,** *809 (1957)*) has reported the results of carotenoid analyses of tissues from eight men, four women and one boy. These subjects were reported to be in apparent good health until immediately before death. The results therefore should represent normal values for carotenoids in man. The fat depots were found to contain 85 per cent of the stored carotenoids; abdominal wall depot fat contained an average of 8.4 micrograms of total carotenoid per gram of lipid. The tissue containing the next highest amount of carotenoids was liver, with an average concentration of 6.3 micrograms per gram of fresh tissue. Smaller quantities of carotenoids were found in other tissues tested although the concentration in the adrenals was quite high.

### Recent Nutrition Books

*Swine Feeding and Nutrition.* Volume 1. T. J. Cunha. Interscience Publishers, Inc., New York. Pp. 278. Price $5.00.

*A Textbook of Dairy Chemistry.* Third edition revised. Volume 1, Theoretical; Volume 2, Practical. Edgar R. Ling. Philosophical Library, New York. Price $12.00 per set.

*La Nutricion en el Paraguay.* Francisco A. Montalto. Impresora Oeste, Buenos Aires. Pp. 447.

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